Marine Pelagic Cyanobacteria: *Trichodesmium* and other Diazotrophs

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Marine Pelagic Cyanobacteria: *Trichodesmium* and other Diazotrophs

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Dedication

This book is dedicated to our doctoral degree mentors: John E. Hobbie (EJC), Barrie F. Taylor (DGC), and Francois M.M. Morel (JGR). Without their patience, guidance and inspiration, this volume, and our careers, would still be dreams.

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PREFACE

Trichodesmium is unique in that it is the only diazotroph, which, lacking heterocysts, is able to fix nitrogen in the daylight under apparent aerobic conditions. It is an interesting organism to work with, partly because of its unique biological properties and partly because of the key role that it plays in nutrient cycling in tropical seas. Because it lacks heterocysts, and because cultures were not available for exchange, some investigators did not believe initial reports of N_2 fixation in spite of the fact that measurements were made using ^{15}N. It was thought that either the data were an artifact, or perhaps associated bacteria were responsible for the fixation of nitrogen. Because of the lack of vigorous culture material, which could be exchanged by researchers, for many years this organism was out of the mainstream of cyanobacterial research and was pretty much in the province of biological oceanographers. While this may have hindered rapid advances *in vitro*, in reality it has been a blessing because it advanced a better understanding of how it behaved in nature, since it forced many individuals (many who would not normally go to sea) to study freshly-collected samples.

There seems to be a continuous stream of surprises associated with *Trichodesmium*. A few of the recent discoveries are: the presence of at least three filamentous cyanobacterial forms regularly associated with it (one of which possesses nitrogenase); a diel pattern of nitrogenase synthesis and degradation; a pattern of diel vertical migration; and the presence of a neurotoxin in *T. thiebautii*.

Many of the advances in understanding it today are being made because of the application of new technologies such as remote sensing, microprobes, molecular biology, plus the fact that cultures are now present in several labs. Because of these recent advances, a NATO-sponsored meeting was convened in Bamberg, Germany, the Franken city of cathedrals and rauchbeer, May 26-31, 1991. We exchanged our results and ideas and planned future research on this and other enigmatic phytoplankters.

This volume is a product of the Bamberg meeting. In addition to *Trichodesmium*, we also discussed the Baltic Sea blooms of *Nodularia* and *Aphanizomenon*, and this led to significant cross fertilization between cyanobacteriologists working in cold and warm waters.

This book is a look at the present and future research on these cyanobacteria. Sometimes, however it is interesting to take a look at the past. A delightful description of a bloom in the Red Sea, which was undoubtedly *Trichodesmium*, was written by E. Dupont in a letter in 1843 and was previously printed in French by Wille (1904).

> "On the 15th of July (1843), the blazing sun of Arabia woke me abruptly, shining suddenly on the horizon without any dawn and in all its splendor. I leaned my elbows rather mechanically on a windowsill at the stern of the ship to seek a vestige of fresh night air before the heat of the day devoured it. What was my surprise to see the ocean astern of the ship colored red as far as the eye could reach! I ran up on the deck and saw the same phenomenon on all sides. I questioned the officers concerning it. The ship's surgeon claimed that he had already observed this occurrence, which, according to him, is produced by the spawn of fish floating at the surface. The others said that they did not recall

having previously seen it. All seemed surprised that I attached such an interest to it. If I had to describe the appearance of the sea, I would say that its surface was covered on all sides by a dense but not very thick layer of a fine material, in color brick-red with a little orange; the sawdust of wood of this color, for example mahogany, would produce approximately the same effect. It seemed to me, and I said so at the time, that it is a marine plant: no one shared my opinion. I had one of the sailors obtain a certain amount of the material by means of a bucket attached to a rope; then with a spoon, I put it into a bottle of clear glass, thinking that it would be better preserved in this way. The next day the material had turned dark violet and the water had taken on a pretty pink color. Believing at the time that immersion would hasten decomposition instead of hindering it, I poured the contents of the bottle on a piece of cotton cloth (the one that I have sent you); the water passed through and it adhered to the cloth. In the process of drying, it turned green, as you presently see it. I should add that on the 15th of July we were broadside of the Egyptian town of Cosseir, that the sea was red during the entire day, that it was the same the following day until around noon, the time that we were in sight of Tor, a small Arab town of which we could see the palm trees in an oasis at the edge of the sea, below a chain of mountains which extends down from Sinai almost to the sandy beach. A little after midday on the 16th, the red disappeared and the surface of the sea became blue again as it was before. On the 17th, we dropped anchor at Suez. The red color consequently was in view from about 5:00 AM on the 15th until about an hour after mid-day on the 16th, that is to say, during a period of 32 hours. During this period, the steamer, making 8 knots, as the saylors put it, covered a distance of 256 miles or 85 1/3 leagues."

This dramatic description of a bloom illustrates some of the fascination associated with *Trichodesmium*. Almost 150 years after Dupont's observations, we note from his description, the rapid onset of the bloom, its accumulation in a layer near the surface, its dark red color, the loss of what may have been phycoerythrin from the collected colonies and the fact that the bloom was extensive and occurred under very warm conditions with high insolation. The bloom color and extent lends credence to the notion that the Red Sea derived its name from *Trichodesmium*, an idea also shared by Ehrenberg. Ehrenberg collected this organism while on a sojourn at Tor near Mt Sinai in December 1823. He stated: "On December 10th I observed there the astonishing phenomenon of the bloody coloration of the entire bay, which forms the harbor at Tor. The open sea outside of the coral reef which shuts off the harbor was colorless as usual. The short waves of the calm sea conveyed to shore a slimy mass which appeared blood-red in the sunlight and was deposited in the sand of the beach, so that at ebb tide the entire bay, which takes a good half hour to cross, was given a blood-red border". He gathered some and further stated: "In the glass containers gathered around me, I noted that the flakes (colonies) all collected on the surface of the water under conditions of sunlight and the warmth of the day. At night, when the containers were shaken, they went to the bottom. After some time, however, they again returned to the surface." Interestingly, it seems that Ehrenberg might have been observing diel changes in the buoyancy of *Trichodesmium*, a phenomenon only recently described from field samples collected in the Bahamas and noted in this volume.

We have come a long way since these early observations; however, there are still a number of major unresolved questions concerning *Trichodesmium* and, for that matter, the Baltic Sea

cyanobacteria. How does *Trichodesmium* protect its nitrogenase from oxygen deactivation? What are the factors which promote blooms? What is the fate of fixed C and N in blooms and the global significance of these planktonic cyanobacteria? What are the biological and genetic differences in species of *Trichodesmium* within and among different regions of the world's oceans? What is the nature and significance of the neurotoxin associated with *T. thiebautii* ? What role do viruses play in terminating blooms? Do the several species of filamentous cyanobacteria associated with *Trichodesmium* colonies affect its biology? How can we best detect cyanobacterial blooms using remote sensing techniques? Why is the DNA of *Trichodesmium* so heavily methylated? The list can go on and on.

Fascination with these colonial cyanobacteria stems from the fact that they are very important in cycling of elements in the sea and are some of the few phytoplankters that one can easily isolate for physiological studies while on board ship. Hopefully this book will stand as a benchmark of where we are today as regards research on pelagic filamentous cyanobacteria and will stimulate further research in the coming decades.

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OVERVIEW OF THE ADVANCED RESEARCH WORKSHOP ON BLOOM-FORMING MARINE CYANOBACTERIA

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ABSTRACT. Blooms of planktonic cyanobacteria occur in oligotrophic tropical and sub-tropical oceans and in the Baltic Sea. Intense N_2 fixation during blooms may provide large inputs of new N to these waters. However, the temporal and areal extent of the blooms and the quantitative significance to upper ocean C and N cycling, while suspected to be high, remain unknown. Recently, several independent lines of research have concluded that open ocean N_2 fixation, one component of new N input, may have been severely underestimated, and this may have been due to the difficulty in quantifying blooms.

Studies on the ecology and physiology of these bloom-forming cyanobacteria in the Baltic and tropical seas have proceeded with little previous scientific interchange between their respective researchers. The application of modern technologies, including molecular biological techniques and remote sensing (among others) has accelerated the pace of effort in each realm. The impetus for assessing the larger scale implications of N_2 fixation in each system has yielded obvious parallel research themes and common questions. For instance, there is yet no clear consensus on the factors or circumstances leading to bloom formation, or of the fate of recently fixed N in either system.

In view of the recent increase in research, the diverse but often common nature of the investigations, and the wide geographic range of these bloom-forming cyanobacteria, it was timely that a comparison and synthesis of knowledge be made, and that future directions in

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research be charted. Hence, with support from NATO and the US NSF, an Advanced Research Workshop (ARW) was held in Bamberg, Germany, from May 26 to May 31, 1991 with the objective of discussing the comparative ecology and physiology of bloom-forming N_2 fixing cyanobacteria in the sea.

The ARW brought together about 40 researchers from twelve countries to review and compare results from their respective areas and to develop an integrative framework for future coordinated research. The meeting promoted unique scientific interactions and exchanges across disciplines (molecular to megascopic levels) and between systems (tropical and temperate cyanobacteria), helped identify common questions, and stimulated cross-fertilization of ideas and approaches.

1. Introduction, Rationale for the Workshop

A comprehensive understanding of N_2 fixation in the pelagic zone of the oceans is essential to our broader understanding of the marine and global nitrogen (N) cycle and oceanic productivity. An accurate assessment of new sources of N entering the euphotic zone provides constraints for estimating the amount of new production (Eppley & Peterson 1979, McCarthy & Carpenter, 1982), and is therefore a means of comparing production estimates based on conventional but often controversial procedures. For such calculations to have meaning, all sources of new N into the euphotic zone need be recognized and quantified. In situ N_2 fixation is one source of new N; however, it has generally been regarded as a minor factor in satisfying planktonic N demands on a global basis (Ryther & Dunstan 1971, Capone & Carpenter 1982). Several hypotheses (e.g. Howarth & Cole 1985, Howarth et al. 1988) have been advanced to explain the apparent "dearth" of marine N_2 fixers. These hypotheses are controversial (Paerl, 1989) and have yet to be rigorously tested in the system where N limitation is of most importance, i.e. the oligotrophic waters of the open ocean.

However, recent evidence suggests substantial underestimation of the contribution of known pelagic diazotrophs (Legendre and Gosselin 1989, Martinez et al. 1983). In some locations (Caribbean Sea, Baltic Sea, Indian Ocean and Arabian Sea), N_2 fixation by planktonic microorganisms can be intense and important relative to N requirements of the phytoplankton population (Carpenter & Price 1977, Carpenter 1983a).

Undersampling of the known open-ocean N_2 fixers may account for some of the perceived underestimation. In particular, several planktonic marine diazotrophs form massive blooms. In tropical and sub-tropical seas around the world, the colony forming non-heterocystous marine cyanobacterium, *Trichodesmium*, is abundant and is known to form areally extensive blooms at times (Karl et al. 1990, Devassy 1987). Dense blooms of *Nodularia* and *Aphanizomenon* also occur in the Baltic Sea. Recently, other unicellular diazotrophs have also been isolated, but their importance is not yet known (Waterbury et al. 1988).

With respect to nitrogen input, *Trichodesmium* has previously been estimated to be responsible for at least one quarter (4.7 Tg) of the nitrogen fixed in the sea annually (Capone and Carpenter 1982). However, relative to oceanic nitrogen requirements by primary production, present estimates of N_2 fixation by *Trichodesmium* only account for about 3.3% of demand for "new" nitrogen (Capone 1991). This extrapolation was based strictly on non-bloom densities over the global zones where *Trichodesmium* occurs (Carpenter 1983) and may therefore be viewed as a lower limit. In the Baltic, N input from *Nodularia* N_2 fixation, including contributions from blooms, has been estimated to account for 0.13 Tg N y^{-1}, or 17% of the total nitrogen input to the

system (Larrson et al. 1985). These extrapolations were based on limited sampling in time and space.

Information on N_2 fixation, besides being of value in further unveiling the complexities of the marine N cycle, also provides new information on the clearly unique N_2 fixing systems of the sea for comparison with their intensively studied terrestrial counterparts. For instance, among N_2-fixing cyanobacteria, *Trichodesmium* is a striking enigma in that N_2 fixation is obligately linked to photic periods of active photosynthesis, although it lacks the cellular adaptation of heterocysts (Capone et al. 1990). It is still unclear how it protects its nitrogenase from O_2 inactivation (Carpenter et al. 1990). Thus, besides their potential importance in open ocean N cycling, planktonic N_2 fixing diazotrophs are important model systems for basic studies in N_2 fixation.

Research on planktonic N_2 fixation in the sea has been, for the most part, conducted by two distinct groups of researchers over the last few decades. Focus has been on either *Trichodesmium* in tropical seas or on *Nodularia* in the Baltic. There has been relatively little interchange between these efforts. Furthermore, owing to the geographically widespread nature of the research on *Trichodesmium*, regional groups have developed which do not often come together. There is much common ground among these groups and efforts, and mutual benefit to be gained by comparing and exchanging information among researchers involved in these respective "systems".

Application of approaches from immunology, sub-cellular physiology, molecular biology and remote sensing is currently fueling intense reexamination of pelagic marine N_2 fixation. This research activity should allow us to resolve some of the recurring and pressing questions which have previously gone unanswered with respect to N_2 fixation in the seas and oceans. Indeed, there have been sufficient recent advances in studies of these natural populations of N_2 fixing plankton to provide for fruitful cross-fertilization of ideas and approaches.

2. Scope of Workshop

The Workshop consisted of eight sessions each with several invited keynote speakers, followed by open discussion. Specific presentations at the workshop focused on questions of biochemistry, genetic regulation, cell ultrastructure, cell physiology, colony morphology, population dynamics as well as community and ecosystem level processes. A poster session provided a forum for specific research efforts of many of the participants.

2.1. SESSION 1 - ULTRASTRUCTURE AND MOLECULAR BIOLOGY

The keynote speaker, Bergman, reviewed recent advances in ultrastructure and enzyme localization in *Trichodesmium*. Colonies were shown to at times be consortia of *Trichodesmium*, other cyanobacteria, eubacteria, diatoms, fungi as well as protozoa and copepods. Subcellular structures were characterized, many of which are well known in other cyanobacteria. Others, such as "scroll bodies", appear to be unique and have no known function. Through enzyme localization techniques, the dynamic nature of the nitrogenase complex and association of the phycocyanin and phycoerythrin pigments with the thylakoid membranes was shown. The localization of the Fe-protein subunit of the nitrogenase complex in specific cells demonstrated intra-colony differences between filaments that may be important in the physiology of these organisms.

The efforts to make restriction maps of portions of the genome from *Trichodesmium* and the use of PCR to isolate the *nifH* gene (coding for the Fe-protein from the nitrogenase complex) were described by Zehr. The diel pattern of expression for nitrogenase and oxygen effects on modification of the nitrogenase enzyme complex was noted.

In the discussion that followed, an observation that modification of the Fe-protein of nitrogenase occurs in cultures of *Trichodesmium* was offered by Ohki. There was a general discussion on the description of the various species of *Trichodesmium*. Although most people use the same morphological criteria to differentiate the major species of *Trichodesmium* (*T. thiebautii, T. erythraeum, T. contorta* and *T. hildebrandtii*), it was agreed that molecular techniques would be very helpful in providing a firmer basis for taxonomy and phylogeny.

2.2. SESSION 2 - NITROGEN UPTAKE AND METABOLISM

The general pathways for N in cyanobacteria and their linkage to photosynthesis were described by Stal. The importance of active uptake for nitrate and the roles of N storage in cells was shown. An example of N_2 fixation in *Gloeothece*, that was simultaneously light and N limited, was provided. Because the cost for heterocyst differentiation and synthesis is so high, it was argued that heterocystous cyanobacteria are only favored in conditions of high light and extremely low external nitrate supply. The non-heterocystous forms, in contrast, are favored under conditions of lower available energy.

The role of glutamine synthetase in N_2 fixation metabolism and the requirement for the Mo containing cofactor were discussed by Rai. He noted the manner in which N_2 fixing cells may cycle ammonium and how the thylakoid membranes may be involved in uptake of ammonium which is assimilated by glutamine synthetase.

Glibert showed that $^{15}NO_3$ is only taken up in late afternoon or in the dark by *Trichodesmium* colonies and $^{15}NH_4^+$ was only assimilated at night. Saino's data which demonstrated *Trichodesmium*'s preference for N source in the order; urea > NH_4+ > NO_3- > N_2 was recalled by Fujita. It was pointed out by Gallon that the ratio of glutamine to glutamate may be an indicator of N sufficiency and that the presence of the N storage product, cyanophycin, during N_2 fixation, indicates N sufficiency.

2.3. SESSION 3 - CARBON AND OXYGEN METABOLISM

A general overview of the complex relationships and interactions of O_2 with photosynthesis in cyanobacteria was provided by Kana. Gallon followed with a discussion of the relationships of nitrogenase and O_2 in non-heterocystous cyanobacteria.

Oxygen protection mechanisms were the focus of the discussion that followed. Three levels of protection; trichomes in the colony, intracellular and molecular were noted by Fujita. Capone reported that while *Trichodesmium* nitrogenase is sensitive to O_2 in natural colonies, there is also evidence of an O_2 requirement under certain situations. Gallon found that *Gloeothece* has an O_2 requirement for N_2 fixation and that it peaks at 10% O_2. Kana explained that the Mehler reaction could only balance the O_2 produced but it could never decrease the O_2 in the cells. Walsby responded to the general question of O_2 diffusion with an appeal to rigorously consider the permeability of gas in cyanobacteria.

2.4. SESSION 4 - CULTURE AND NUTRITION OF *TRICHODESMIUM*

The importance of iron nutrition in many metabolic processes was reviewed by Rueter. He noted that the iron requirement for *Trichodesmium* and other N_2 fixing cyanobacteria is approximately ten times as high as in non-N_2 fixing cyanobacteria. The probable source of iron to *Trichodesmium* in the open ocean was aeolian-transported particulate "dust", and *Trichodesmium* may have mechanisms for capturing and leaching this dust. The discussion that followed focused on culture techniques and iron nutrition. Rueter commented that the ability to culture *Trichodesmium* is not a simple matter because he has used his media and techniques at NIBB with Ohki and Fujita but has never been able to grow *Trichodesmium* at his own lab in Oregon. Ohki described the stable cultures that she has at NIBB. Letelier also has *Trichodesmium* in stable culture at Hawaii, and he gave details on the isolate.

2.5. SESSION 5 - FACTORS CONTRIBUTING TO BLOOM FORMATION

The importance of gas vesicles in buoyancy regulation was discussed by Walsby. The protein structure of the vesicles and the genes that code for these have been defined in some species. The amount of pressure that it takes to collapse the vesicles was related to the maximum pressure typically experienced by the cyanobacterium in its natural environment.

Data on the vertical distribution of *Trichodesmium* in the water column, were presented (Villareal). Carbohydrate ballast accounted for part of the change in buoyancy. Limitation by phosphate increases vertical migration in some species. Eukaryotic algae also migrate vertically, and this migration may help utilize spatially separated sources of light and nutrients.

Factors that lead to bloom formation including physical stability, high pH, and colony shape were discussed by Paerl. He defined a bloom as a perceptible change in water quality or a dynamic increase in population density.

The results of a mathematical simulation based on the buoyancy as determined by gas vesicles and carbohydrate ballast were presented by Kromkamp. Means by which colony movement could cycle or converge at a depth was demonstrated. The discussion that followed focused on the various definitions of blooms. It was noted that blooms of picoplankton are undetectable by any easily measured characteristic. The size of the colony as it relates to self shading and potential Stokes velocity was considered.

2.6. SESSION 6 - FATE OF BLOOMS

A model for community dynamics in the presence of a bloom was given by Sellner. The "microbial loop", the consumption of organic matter and release of dissolved nutrients by heterotrophic microorganisms, plays a dominant role in these communities. In *Trichodesmium* blooms, this loop may be accelerated by organics leaking from *Trichodesmium* colonies or by the disruption of the colonial consortia. Grazing usually plays a major role in bloom dynamics but is poorly understood with respect to *Trichodesmium.*

The toxins that are made by several species of cyanobacteria were described by Codd. In freshwater blooms these toxins can cause many problems including closure of water bodies to public use. *Trichodesmium thiebautii* also contains toxins, but so far, *T. erythraeum* does not. During the discussion, O'Neil described some observations on the grazing and the sensitivity of several copepods to these toxins.

2.7. SESSION 7 - CYANOBACTERIAL BLOOMS IN THE SEAS AND OCEANS

Nodularia and *Aphanizomenon* summer blooms in the Baltic Sea were described by Boje. The maximum nitrogen fixation rate in the Baltic was proportional to surface water temperature. Conditions that seem to be required for these blooms are a low N/P ratio, availability of P, a shallow mixed layer, low grazing and a seed population. Destruction of the bloom occurs with strong wind or surface cooling.

An extensive bloom of *Trichodesmium* was observed by Karl in the North Pacific near Hawaii, and the estimated N_2 fixation appeared to be important in the N and P budgets of the oceanic gyres. Karl suggested that *Trichodesmium* may fix more N_2 than needed for its own growth.

In the discussion that followed, the excess N produced by *Trichodesmium*, termed "over production", was related to potential for P regeneration within the colony and to the requirement that N and P metabolism must be uncoupled to some extent. No polyphosphate granules have been observed yet in natural samples of *Trichodesmium*. The abundance of *Trichodesmium* and the potential N_2 fixation rate indicates that this is important as a source of new N for the euphotic zone.

2.8. SESSION 8 - REMOTE SENSING OF BLOOMS

Satellites that may be useful for the detection of *Trichodesmium* were described by Gower. In particular four bands of CZCS data can be used to look for bright reflectance blooms with a chlorophyll signature. Examples of this have been seen near the Great Barrier reef, off of the Indian sub-continent, and off Kenya. Self-shading in the water column is an important parameter in limiting the ability to detect the density of a bloom. Borstad elaborated on the red edge effect for plants near the surface of the water and how the signature of *Trichodesmium* varies from diatoms.

The Baltic system has a high anthropogenic input of phosphorus and also receives a large input of atmospheric ammonium from cows and cars on the surrounding land (Horstman). Historically, the primary productivity of the Baltic Sea has increased since 1950. Remote sensing is useful in locating blooms, but the algorithms need to be adjusted carefully. Methodology for calculating chlorophyll-a from CZCS imagery was discussed by Dupouy, and the correlation coefficient of this correction changes with time because of variations in the sensors and other factors. She noted the occurrence of yellow blooms in satellite images in the summer near New Caledonia. Other blooms in the area have characteristic red or white signatures which indicate the importance of pigments and reflection in determining the signatures of surface populations.

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TRICHODESMIUM: ULTRASTRUCTURE AND PROTEIN LOCALIZATION

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ABSTRACT. Colonial morphology, associated organisms, and ultrastructural organization of *Trichodesmium thiebautii* cells were investigated in detail using light, scanning and transmission electron microscopy. The ultrastructure of *Trichodesmium* is quite different from that of other cyanobacteria. *T.thiebautii* can be differentiated from *T. erythraeum* on the basis of colonial morphology and the subcellular arrangement of inclusions. The main differentiating features are highlighted. A variety of associated organisms were observed in the colonies including two additional cyanobacteria. The presence and distribution of proteins involved in carbon and nitrogen metabolism and their regulation in *T. thiebautii* are also briefly discussed.

1. Introduction

Cyanobacteria possess a prokaryotic subcellular structure. The ultrastructure of about 200 cyanobacterial species has so far been described, and 32 inclusion bodies have been identified (Jensen 1990). Literature pertinent to the ultrastructure of the marine planktonic cyanobacterium, *Trichodesmium* is fragmentary, and only two (*T. thiebautii* and *T. erythraeum*), out of the four marine species (Carpenter 1983), have been investigated. The initial studies on *T. erythraeum* by van Baalen and Brown (1969) and Bryceson and Fay (1981) were followed by investigations on *T. thiebautii* by Gantt *et al.* 1984, Haxo *et al.* 1987 and Li and Lee 1990. Recently attempts to identify and localize structures such as thylakoids and carboxysomes were made using immunogold labeling and TEM (Siddiqui *et al.* 1991a).

Species in the genus *Trichodesmium* are unique in that they are the only known non-heterocystous cyanobacteria which can fix nitrogen during the day under apparently aerobic conditions. In addition, *Trichodesmium* appears to be the major diazotroph in the pelagic zone of the world's oceans. Nitrogen fixation, therefore, has been the most studied aspect of *Trichodesmium* 's physiology (Carpenter 1983, Fogg 1987).

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We are currently investigating *Trichodesmium* with regard to colonial morphology, associated organisms, subcellular structures and their diel variations, as well as the distribution and localization of key proteins involved in carbon and nitrogen metabolism. The aim of this article is to highlight our recent research on these aspects in *T. thiebautii* and to compare results, when apropriate, with previous reports. As recent research on nitrogenase activity (Capone *et al.* 1990) and the distribution of proteins such as nitrogenase (Paerl *et al* 1989, Bergman and Carpenter 1991) and ribulose 1,5-bisphosphate carboxylase/ oxygenase (Rubisco) (Siddiqui *et al.* 1991a), as well as a reevaluation of oxygen protection of nitrogenase (Carpenter *et al.* 1990), have drastically modified the previous concepts of nitrogen and carbon metabolism in *Trichodesmium*, these aspects are also briefly discussed.

2. Material and Methods

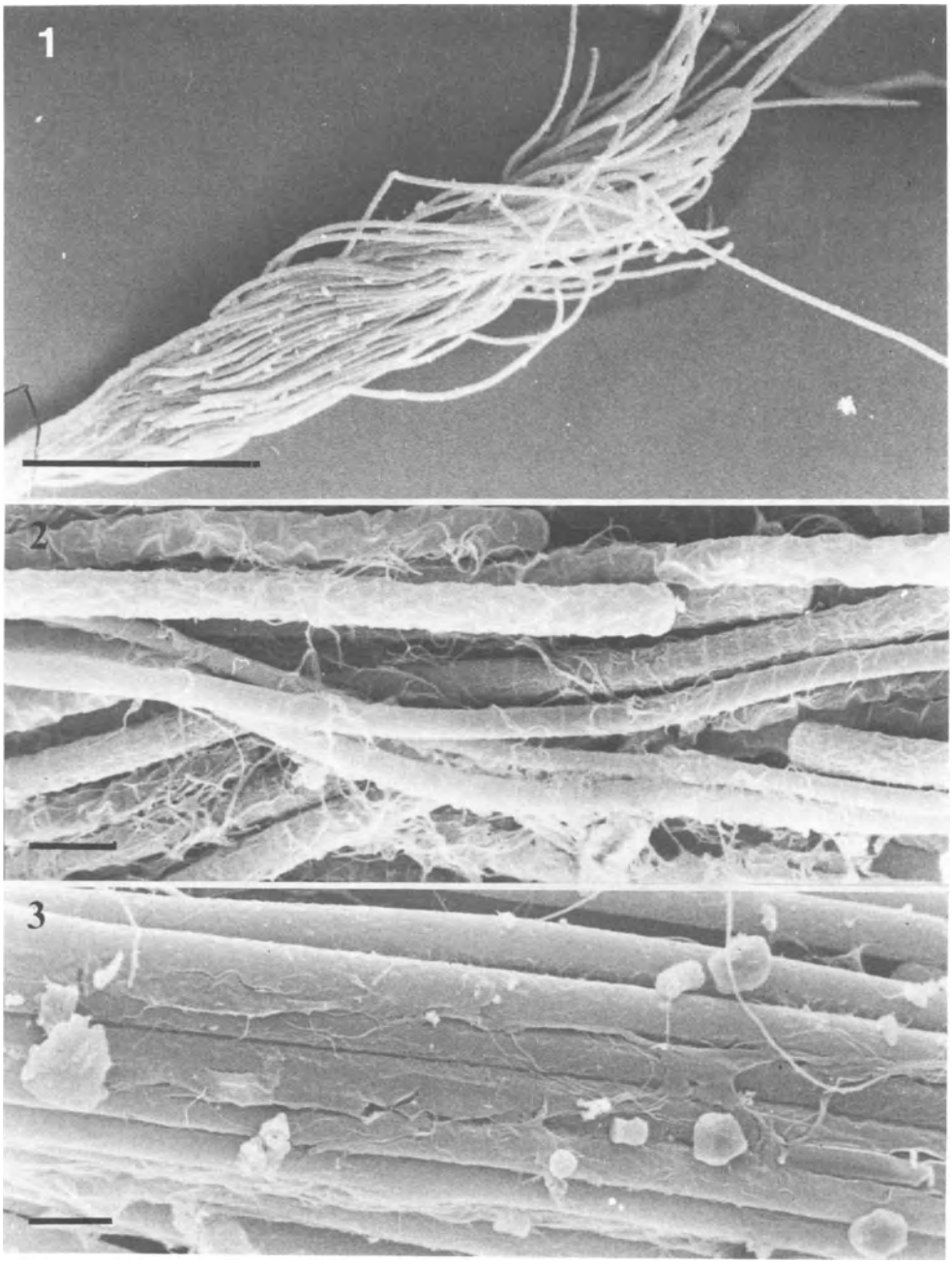
Morphological and ultrastructural investigations were made, with the help of light (LM), scanning (SEM) and transmission electron microscopy (TEM). Colonies of *T. thiebautii* were collected from the Sargasso Sea as described earlier (Bergman and Carpenter 1991, Siddiqui *et al.* 1991a,b). For SEM studies colonies were fixed in glutaraldehyde (2.5%) and dehydrated in a concentration series of ethanol (20-100%) and acetone (100%). Later, following critical point drying (CPD 020 Blazer Union Ltd.) and gold sputtering (SEM Coating Unit E% 100, Polaron Equipment Ltd.), specimen were viewed in a SEM (Cambridge Stereoscan 10). For ultrastructural studies colonies were fixed in glutaraldehyde (2.5%), and post-fixed in osmium tetroxide (2%) as described previously (Siddiqui *et al.* 1991a,b). Ultrathin sections on copper grids, coated with formvar and carbon, were observed with either a Jeol 100B or Zeiss 10 TEM.

3. Observations and Discussion

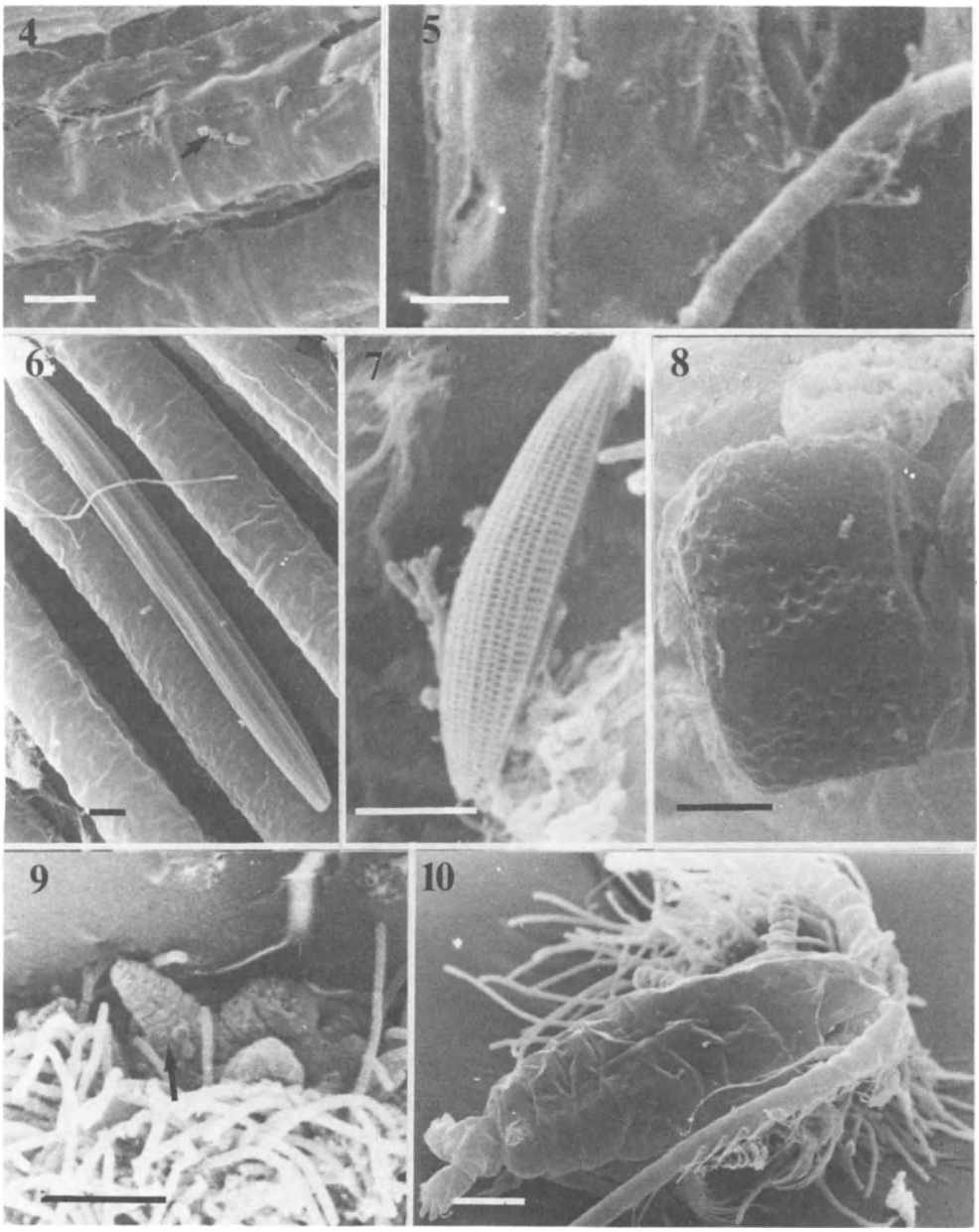
3.1 THE *TRICHODESMIUM* COLONY

3.1.1. *Morphology. Trichodesmium* colonies are aggregates of a few to several hundred trichomes. The trichomes are aligned either in parallel, resulting in a fusiform colony (tufts) or arranged radially giving rise to a spherical form (puffs). Fusiform colonies of *T. thiebautii* are the dominant species in, for example, the Sargasso Sea, followed by the radial forms. The fusiform colonies of *T. erythraeum* are typically less abundant than *T. thiebautii*. Although colonial morphology has been considered as an insufficient basis for the identification of *Trichodesmium* species (Geitler 1932), the fusiform colonies can conveniently be distinguished into flat or raft-like *T. erythraeum* and rope-like twisted *T. thiebautii* colonies (Nagasawa and Marumo 1967, Desikachary 1959). Desikachary (1959), despite mentioning the presence of the radial *Trichodesmium* colonies in nature, did not consider these forms in his description of species. Radiate colonies have, however, been identified as both *T. thiebautii* (Nagasawa and Marumo 1967, Walsby 1978, Gantt *et al.* 1984, Li and Lee 1990) and *T. erythraeum* (Bryceson and Fay 1981). The basis for identification in these reports is not known. In order to avoid discrépancies in the taxonomy of *Trichodesmium* species and the usage of nomenclature, and to be able to correlate data with the correct species, further investigations are highly desirable.

3.1.2. *Associated Organisms.* Several other prokaryotic and eukaryotic organisms have been reported to be present in *Trichodesmium* colonies (Carpenter 1983, Siddiqui *et al* 1991b) (Figs. 4 to 10). Besides, e.g., bacteria, fungi, diatoms, protozoans, tunicates, hydrozoans, and copepods, we have recently identified three frequently occurring filamentous organisms (Siddiqui *et al.* 1991b), two of which are cyanobacteria. These are provisionally classified under the LPP-group (*Lyngbya, Phormidium* and *Plectonema*). The only other report on the occurrence of associated cyanobacteria is by Wille (1904) who described a unicellular and a filamentous cyanobacterium in association with *Trichodesmium* colonies.



Figures 1-3. Scanning electron micrograph of *Trichodesmium* colonies. Fig. 1, overview of a typically twisted (rope-like) *T. thiebautii* colony. Bar = 0.5 mm. Fig. 2, detail of *T. thiebautii* colony. Trichomes are irregularly bent and vary in width. Note the numerous associated organisms among the trichomes. Bar = 20 μm. Fig. 3, detail of *T. erythraeum* colony. Trichomes are straight, parallel and uniform in size. Bar = 20 μm.



Figures 4 - 10. Scanning electron micrograph of a number of prokaryotic and eukaryotic organisms associated with *Trichodesmium* colonies. Fig. 4, rod shaped bacterial cells attached to a *Trichodesmium* trichome (arrow). Bar = 5 μm. Fig. 5, the recently described cyanobacterial filaments. A large (about 1 μm) and a small (0.5 μm) thin cyanobacterial filaments are shown. Bar = 2 μm. Fig. 6 and 7, species of pennate diatom. Bar = 5 μm. Fig. 8, a centric diatom species. Bar = 5 μm. Fig. 9, part of radial colony harbouring a hydroid, *Pelagiana trichodesmiae*. Note the partially contracted polyps (arrow). Bar = 100 μm. Fig. 10, a copepod apparently feeding on a radial *Trichodesmium* colony. Bar = 100 μm.

Consortia of such diverse taxonomic groups makes *Trichodesmium* colonies an unusual entity, and the biological relations between these organisms demand further study. *Trichodesmium*, besides being a primary producer and the major species incorporating new nitrogen in the euphotic zone, may provide buoyancy, shelter and food to the other organisms and hence extend the chance for their survival and growth.

3.1.3. *Cellular Differentiation.* Trichomes in a colony of *T. thiebautii* vary considerably in cell width, from 6 to 20 μm (Fig. 2), whereas *T. erythraeum* cells have rather consistant diameter (Fig. 3). Our own TEM observations on longitudinal sections of *T. thiebautii* showed a variation in the compactness of intracellular inclusions of the filaments from those being quite dense to those being

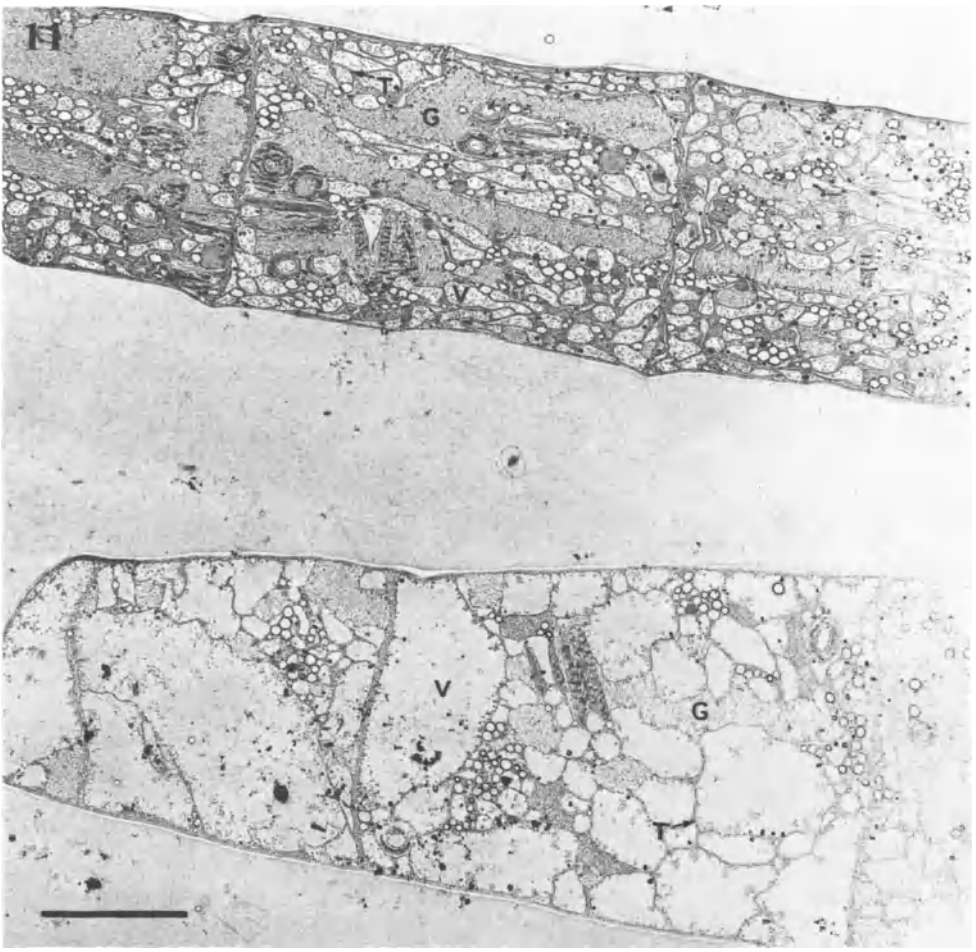


Figure 11. Part of longitudinally sectioned *T. thiebautii* colony in TEM depicting the subcellular differences between trichomes. Some trichomes may be rather compact (top) with narrow cells possessing numerous inclusion bodies and some more vacuolated (bottom) with wider cells containing larger vacuole-like structures (V), and fewer gas vacuoles (G), thylakoids (T) and other inclusions. The vacuolated filaments possibily represent a different growth stage. Bar = 5 μm.

highly vacuolated. Vacuolated cells were always larger and possessed fewer inclusion bodies (Fig. 11). We suggest that the vacuolated filaments are older because some of these have disrupted membranes and distorted subcellular organization. Our finding is in contrast to a recent report on the same species (Li and Lee 1990) where the presence of three types of cells: terminal vacuolated, central homogeneous, and granular, were noted. For *T. erythaeum* the trichomes have been proposed to be divided into granulated and non-granulated regions of trichomes (Bryceson and Fay 1981), and this was putatively related to the presence and absence of carboxysomes, respectively. Whether the structural differences observed are caused by physiological, functional or growth specific manifestations is not yet clear.

3.2. SUBCELLULAR STRUCTURES

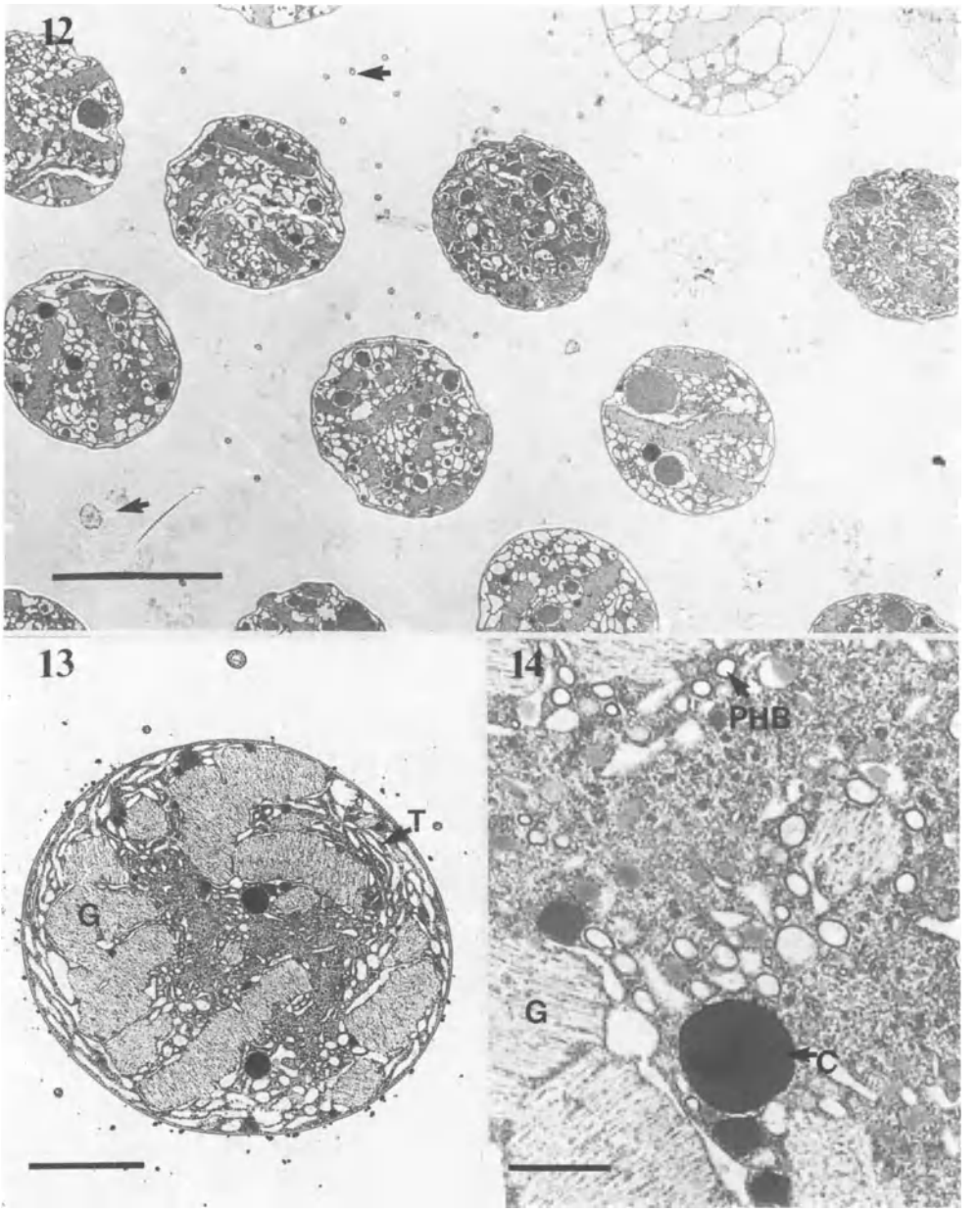
A overview of a transversely sectioned *T. thiebautii* colony in TEM revealed that spaces between *Trichodesmium* trichomes harbour a number of associated organisms (Fig. 12), as discussed above (see 3.1.2). Figures 13 and 14 demonstrate a representative *T. thiebautii* cell and a higher magnification of the cytoplasmic area, respectively. The following passages describe and compare the fine structure of subcellular compartments identified so far in *Trichodesmium* species.

3.2.1. *The Cell Wall. Trichodesmium* possesses a typical gram-negative cell wall common to cyanobacteria (Fig. 15). The total thickness of the cell wall in *T. thiebautii* is about 70 nm. Proceeding from the plasmamembrane towards the outside, first an electron transparent zone about 10 - 13 nm (10 nm in *T. erythraeum*; van Baalen and Brown 1969) is present. Next in order is an electron opaque peptidoglycan layer of about 22-26 nm. The thickness of this layer in *T. thiebautii* is lower than that observed by Haxo *et al.* (1987). In *T. erythraeum* a 20 nm wide peptidoglycan layer has been reported (van Baalen and Brown 1969). The peptidoglycan layer is apparently more pronounced in *Trichodesmium* than that of other gram-negative prokaryotes. An extraordinarily thick peptidoglycan layer has previously been found in *Oscillatoria* species (Cohen-Bazire 1988). A third electron dense zone, similar in thickness (10 nm) to the earlier reports on two *Trichodesmium* species, seems to be present just outside the peptidoglycan layer. The outer membrane is 3 nm thick (3 - 5 nm; Haxo *et al.* 1987) in *T. thiebautii*, but it is thicker (7 nm) in *T. erythraeum* (van Baalen and Brown 1969). Our data also confirm the presence of fine intermittent spaces in the outer membrane layer similar to that observed by Gantt *et al.* (1984).

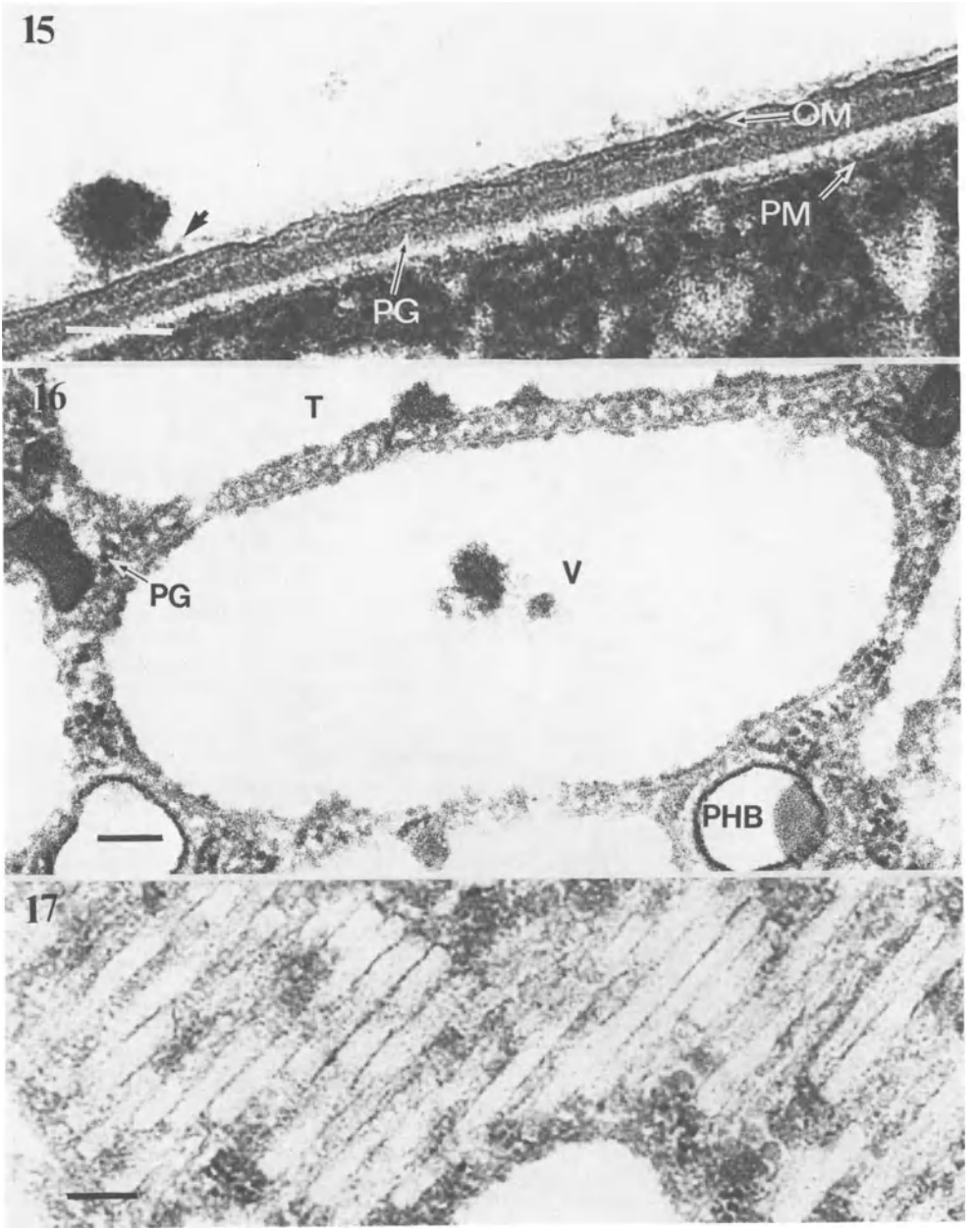
Junctional pores have been reported to be present in the peptidoglycan layer (Haxo *et al.* 1987, Gantt *et al.* 1984) and were considered as cellular connections to the exterior. Gantt *et al.* (1984) proposed these junctions as fracture spots for the initiation of cell lysis rather than the thinning of the peptidoglycan layer (van Baalen and Brown 1969). External to the cell wall a striated sheath layer, also designated as an extra cell wall layer in some other cyanobacteria (Jensen 1990), has been observed in *T. thiebautii* (Gantt *et al.* 1984) (Fig. 15). An outer sheath covering individual trichomes has also been observed with SEM in *Trichodesmium* (Siddiqui *et al.* 1991b).

3.2.2. *Thylakoid membranes.* Thylakoids are sac-like membranous structures present in all cyanobacteria. Attached to these membranes are phycobilisomes containing phycobiliproteins and the light harvesting chromophores. Generally, thylakoids are arranged in parallel in the peripheral region of the cyanobacterial cells, but in *Trichodesmium* they are present throughout the cells and are not stacked. In *T. erythraeum*, these membranes are concentrated in the central core area (van Baalen and Brown 1969). Contrarily, in *T. thiebautii* (Fig.16), they form a loose network (Gantt *et al.* 1984, Li and Lee 1990). Our own observations on *T. thiebautii* from the Sargasso Sea, confirm these reports. However, a radial arrangement of thylakoids, in contrast to all other reports, has also been reported for the same species when held in culture (Haxo *et al.* 1987).

The presence and the distribution of phycobiliproteins, phycoerythrin (PE) and phycocyanin (PC) has recently been examined in *T. thiebautii* using immunocytochemistry and TEM (Siddiqui *et al.* 1991a). This study showed that both PE and PC were present in all cells, and that PE is present in comparatively higher ammounts. This quantitative relationship corresponds to previous observations (Fujita and Shimura 1974). The gold label distribution clearly demonstrated the network configuration of thylakoid membranes. Spectral absorbtion characteristics and the types of pigments, such as, phycobiliproteins, including phycourobilins and phycoerythrobilin, and



Figures 12 - 14. A transversely sectioned *T. thiebautii* colony viewed in TEM. Fig. 12, a typical overview of part of the central area of a colony. A variation in compactness of the cells (compare to Fig. 11) is obvious. A number of other organisms are seen among the trichomes (arrow). Bar = 10 μm. Fig. 13, transverse section of a typical *T. thiebautii* cell. Note the large and irregularly arranged gas vacuoles (G)and the thylakoid network (arrow). Bar = 5 μm. Fig. 14, enlargement of the cytoplasmic area of the cell in Fig. 13, depicting gas vesicles (G), cyanophycin granules (C), possibly poly-β-hydroxybutyric acid granules (PHB), and other cytoplasmic inclusions. Bar = 1 μm.



Figures 15 - 17. Details of *T. thiebautii* cells in TEM at higher magnification. Fig. 15, the cell wall. Plasmamembrane (PM), peptidoglycan layer (PG), outer membrane (OM), and additional sheath material outside the outer membrane (arrow). Bar = 0.1 μm. Fig. 16, part of a cell showing the thylakoid network (T) and the prominent vacuole-like structures (V). Other structures noted are those tentatively identified as PHB granules and polyglucoside granules (PG). Bar = 0.1 μm. Fig. 17, close view of a gas vacuole. Vesicles are longitudinally sectioned revealing their small width and variability in length. Bar = 0.1 μm.

carotenoids in *Trichodesmium* have recently been reviewed elsewhere (Carpenter 1983, Hoffman *et al.* 1990).

3.2.3. *Vacuole-like Structures.* These unusual structures, among cyanobacteria, occur frequently in *T. thiebautii* and (Figs. 11 and 16) are present in all types of cells described so far (Li and Lee 1990, Siddiqui *et al.* 1991a). Highly vacuolated cells are not common among cyanobacteria, and similar structures have only been reported in *Gloeothrichia pisum* (Jensen 1990). However, prochlorophytes also contain such vacuoles (Swift 1989). The vacuoles have been suggested to originate by the dilation of thylakoids and were hence designated as intrathylakoidal spaces in another cyanobacterium (Jensen 1990), in prochlorophytes (Swift 1989), and also in *Trichodesmium* (Haxo *et al.* 1987). The thylakoid membranes, although present in close association with the vacuoles, do not encompass these structures in *T. thiebautii*, and thus the vacuole-like structures appeared to be separate cytoplasmic inclusions. However, the relatedness in space of vacuoles and photosynthetic membranes may suggest a close functional interaction. Vacuoles may serve as storage site for photosynthetic metabolites (as in higher plants) and/or may have a function somewhat similar to vacuoles of the higher plants. There is a diurnal variation in the size and content of these vacuoles (manuscript in preparation).

3.2.4. *Gas Vesicles.* Gas vesicles are hollow cylinders with tapering ends (Walsby 1972). In *Trichodesmium* they are stacked and form bundles referred to as gas vacuoles. The arrangement of gas vacuoles is a characteristic differentiating *T. erythraeum* and *T. thiebautii*. The distribution of gas vacuoles in *T. erythraeum* , located along the periphery of the cells (van Baalen and Brown 1969), differs from that of *T. thiebautii* where they are irregularly arranged throughout the cells (Figs. 11, 13 and 17). About 60-70% of the cell volume is known to be occupied by the gas vesicles in *Trichodesmium* (van Baalen and Brown 1969). The longitudinal axis of the vesicles is perpendicular to the longitudinal axis of the *T. erythraeum* cells (van Baalen and Brown 1969), whereas, vesicles can occur at any angle with respect to the cell axis in *T. thiebautii* (Fig. 18). The cross sectional width of the vesicles is small, about 78 nm in *T. erythraeum* (van Baalen and Brown 1969), and we have observed even smaller gas vesicles, about 45 nm, in *T. thiebautii*. This corresponds to previous observations (Gantt *et al.* 1984). In *Trichodesmium*, the length of gas vesicles is variable (Fig. 17) but their width is generally consistent (Fig. 19). The observations by Haxo *et al.* (1987) on the presence of a limited number of gas vesicles contradict our observations (this report) and others (Gantt et al. 1984, Li and Lee 1990) in *T. thiebautii*.

The vesicles in *Trichodesmium* are extremely strong, and can withstand high pressures, e.g., the mean collapse pressure for vesicles in *T. erythraeum* is about 12 bars and in *T. thiebautii* is as much as 37 bars (Walsby 1978). This, no doubt, gives *Trichodesmium* colonies an extensive ability to withstand high pressures of deep oceanic waters. Walsby (1978) suggested that the turgor pressure collapse of the gas vesicles is not involved in regulation of the buoyancy in *Trichodesmium*. Despite the presence of such strong vesicles, van Baalen and Brown (1969) claimed to have observed collapsed vesicles in *T. erythraeum*. The observation was based on the width of the appressed structures seen among the gas vesicles. We have not observed structures which appear to be collapsed vesicles in *T. thiebautii*.

3.2.5. *Cyanophycin Granules.* The cyanophycin or structured granules are known to function in nitrogen storage in cyanobacteria. They consist of copolymers of aspartic acid and arginine (Simon 1971). Although, numerous cyanophycin inclusions are present in *T. thiebautii*, none have been observed in *T. erythraeum* cells. Invariably all cyanobacteria examined possess cyanophycin except for some species of the unicellular cyanobacterium, *Synechococcus* (Lawry and Simon 1982). In general, the number and the sizes of this inclusion vary with growth rate and environmental conditions in cyanobacteria (Lawry and Simon 1982). However, cells of *T. erythraeum* have high amounts of phycoerythrin, which gives it a dark reddish-brown coloration. It is possible that these proteins may function as nitrogen reserves in *T. erythraeum*. For instance, phycoerythrin has this role in *Synechococcus* spp., a cyanobacterium which also lacks cyanophycin accumulation (Barlow and Alberte 1985). Highly vacuolated cells of *T. thiebautii* also lack cyanophycin (Li and Lee 1990). It may be that when *Trichodesmium* cells age, a depletion of nitrogen reserves takes

place similar to the observed disappearance of cyanophycin granules during the stationary growth phase in other cyanobacteria (Allen 1984).

3.2.6. *Polyhedral Bodies.* Polygonal inclusions are present in all cyanobacteria (Codd and Marsden 1984). These are generally located in the central area of the cells and among the thylakoids. Their size varies from 0.2 μm to 0.7 μm in cross-section. Polyhedral bodies in other cyanobacteria have been discussed in detail by Codd and Marsden (1984), Jensen and Bowen (1961), and Jensen (1990). These bodies contain the key catalyst of carbon fixation, ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco), as a major constituent (Lanaras and Codd 1981) and therefore these structures are termed carboxysomes. Polyhedral bodies are reported in the two species of *Trichodesmium* studied so far. Bryceson and Fay (1981) have proposed the presence of these bodies but only in some cells of *T. erythraeum* and defined these as granulated cells, the rest being non-granulated cells. On the other hand, polyhedral bodies are reportedly present in all cells of *T. thiebautii* (Li and Lee 1990, Siddiqui *et al.* 1991a). Li and Lee (1990) did not detect Rubisco in the polyhedral bodies of *T. thiebautii* using immunogold labeling and an anti-Rubisco antiserum but we have recently obtained evidence for the presence of Rubisco in carboxysomes (Fig. 29) (Siddiqui *et al.* 1991a).

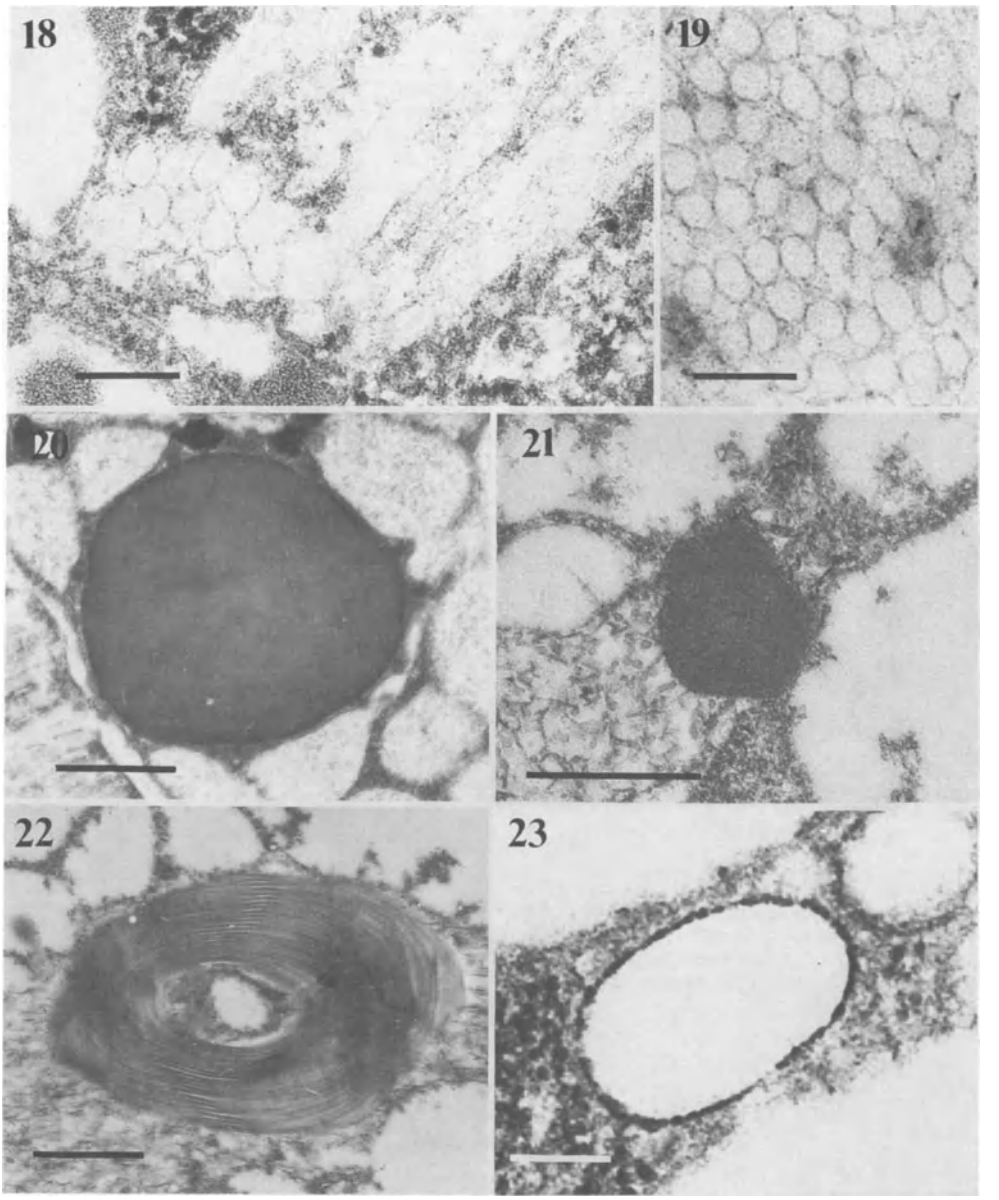
3.2.7. *Multimembranous Scroll-like Structures.* These unusual structures, first reported in heterocysts of *Gloeothrichia pisum* (Jensen and Bowen 1970), have frequently been observed in *T. thiebautii* (Gantt *et al.* 1984, Li and Lee 1990). These bodies are composed of concentrically arranged layers of membranes. In *T. thiebautii* they vary between 1.5 - 1.7 μm in diameter (Fig. 22). Earlier reports indicated a smaller diameter of about 0.4 - 1.0 μm (Gantt *et al.* 1984, Li and Lee 1990). Size variation appears to be related to the degree of lamellation. We have observed about 20-25 concentric layers of membranes contrasting the presence of 10-13 (Gantt *et al.* 1984) or even fewer (Li and Lee 1990) layers in previous studies.

Scroll-like structures are always found in the vicinity of the gas vesicles in *T. thiebautii*. A similar relationship has been noted by Gantt *et al.* (1984). The significance of this physical nearness and the function of these membranes, which differ distinctly from the thylakoid membranes, is not known. However, Jensen (1990) has suggested that they may function in toxin production similar to what has been proposed for scroll-like bodies of Kappa particles (bacteria present in *Paramecium aurelia*). Interestingly, in a recent study the production of a highly potent neurotoxin was observed in *T. thiebautii* but not in *T. erythraeum* (Hawser *et al.* 1991). The lack of scroll-like structures in the latter species may possibly indicate the aforementioned function.

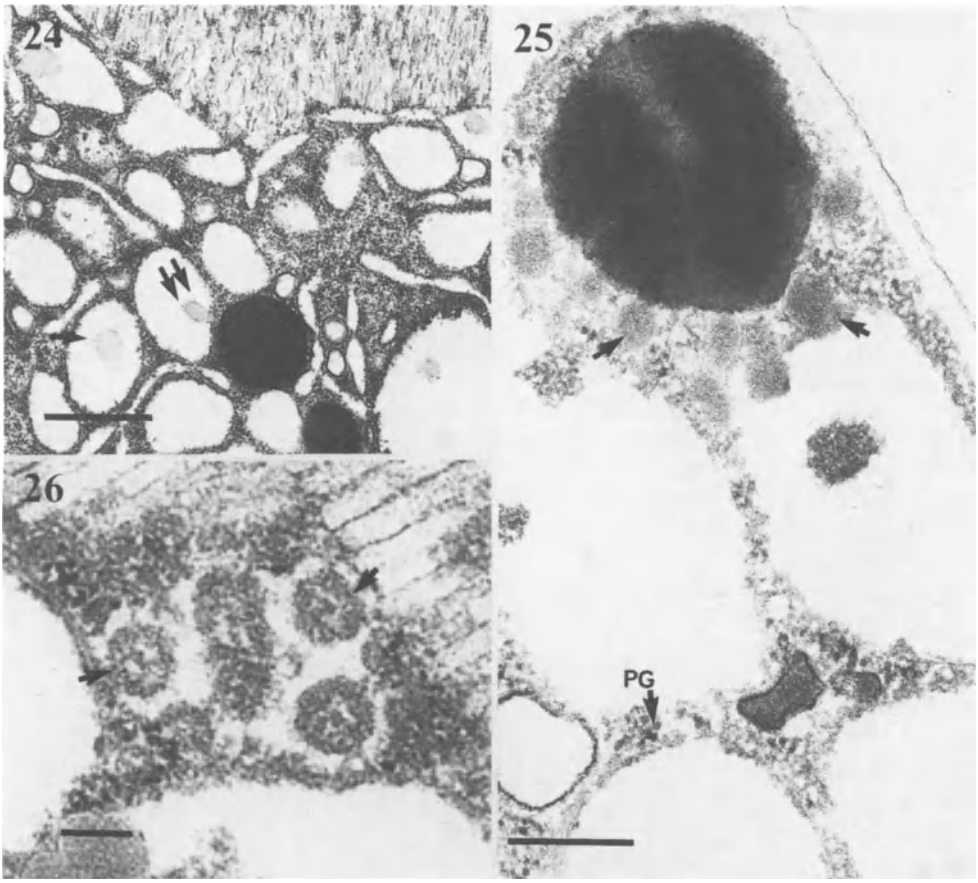
3.2.8. *Poly-β-Hydroxybutyric Acid Granules (PHB).* PHB granules are a widespread inclusion in bacteria and may account for up to 60% of the dry weight of the cells (Shively 1974). Their occurrence in the cyanobacteria is sporadic. The accumulation of PHB polymer has been reported in *Chlorogloea fritschii* (Carr 1966, Jensen and Sicko 1971), in *Anacystis cyanea* (Sicko-Goad 1982), in *Spirulina* spp. (Campbell *et al.* 1982, Vincenzini *et al.* 1990) and in *Aphanocapsa* sp. (Allen 1984). The function of these granules is to store energy and carbon in the cell.

PHB granules have not been reported previously in *Trichodesmium*. Figure 23, however, demonstrates the frequent occurrence of structures, similar in appearance to PHB granules, in *T. thiebautii*. These granules are oval to round structures, delimited by an electron dense monolayer, and typically seen either as empty bodies or as partly filled. Their diameter in *T. thiebautii* is about 0.26 μm. In order to determine the exact nature of these structures in *Trichodesmium* further chemical analyses are required.

3.2.9. *Polyphosphate Bodies.* Accumulation of polyphosphate into distinct subcellular compartment is a regular feature of cyanobacterial cells. They appear as electron-dense bodies often with a porous appearance in the electron microscope. In *Trichodesmium*, polyphosphate inclusions have been observed in *T. erythraeum* (van Baalen and Brown 1969). Despite the fact that polyphosphate bodies are normal constituents of cyanobacterial cells, having a role in storing excess polyphosphates, neither the earlier reports nor our own observations indicate their presence in *T. thiebautii*.



Figures 18-23. Subcellular structures in *T. thebautii* cells in TEM at high magnification. Fig. 18, the orientation of gas vesicles, may occur both in parallel and perpendicular to the vertical axis of the cells. Bar = 0.1 mm. Fig. 19, transverse section through a gas vacuole. Note the consistent diameter of hollow cylindrical vesicles. Bar = 0.1 mm. Fig. 20, cyanophycin granule. Bar = 0.5 mm. Fig.21, polyhedral body (carboxysome). Note the delimiting layer (arrow) around the structure. Bar = 0.4 mm. Fig. 22, close view of a scroll-like multimembranous body. Bar = 0.5 mm. Fig. 23, possibly a poly-ß-hydroxybutyric acid granule. Bar 0.1 mm.



Figures 24 - 26. Subcellular structures in *T. thiebautii* cells in TEM at high magnification. Fig. 24, medium electron dense granule fully inside a vacuole-like structure (single arrows) and the same structure attached to the periphery of the vacuole-like structures (double arrow). Bar = 1 μm. Fig. 25, similar granules (arrow), as in Fig. 24, but now mainly located around a cyanophycin granule. Also note the numerous small granules, possibly polyglucoside granules (PG), in the vicinity of thylakoids. Bar = 0.2 μm. Fig. 26, cylindrical bodies (arrow) in the cytoplasm. Bar = 0.1 μm.

3.2.10. *Medium Electron Dense Granules.* Numerous small medium electron dense granules of amorphous appearance are present in *T. thiebautii*. These are about 0.18 μm in diameter and are associated with the vacuole-like structures (Fig. 24). They occur either completely inside the vacuoles, or in some instances, attached to the peripheral walls of the vacuoles. The size and presence of these granules vary with time of collection, implying that they may serve a short-term storage reserve function. As structures of a similar appearance are also detected dispersed in the cytoplasm around some cyanophycin granules (Fig. 25), in agreement with observations by Li and Lee (1990), may point to their involvement in the nitrogen metabolism. The composition and function of these granules is, however, yet to be resolved.

3.2.11. *Cylindrical Bodies.* Cylindrical bodies are present in both *T. erythraeum* (van Baalen and Brown 1969) and *T. thiebautii* (Gantt *et al.* 1984). Although 1-3 bodies have been reported to be present in a *T. erythraeum* cell, our results on *T. thiebautii* indicate a sparse distribution of this

inclusion (Fig. 26). The cylindical bodies of *T. thiebautii* have a simpler structure than those of *T. erythraeum* (Gantt *et al.* 1984). Gantt *et al.* (1984) have related these structures to the spherical spoke inclusions present in *Calothrix* and *Gloeothrichia* in which they have been proposed to serve as storage of unknown material (Jensen 1990). Cylindrical bodies have also been suggested to play a role in providing reduced microzones within the cells needed for the optimum functioning of nitrogenase, a highly oxygen-labile enzyme (Jensen 1990). The tubular thylakoid membranes produce cylindrical bodies in some cyanobacteria (Jensen 1990), but this does not seem to be the case in the *Trichodesmium* cells as they have thylakoids in sheaths rather than in a tubular configuration (Gantt *et al.* 1984). The origin and the function of these bodies is an open question.

3.2.12. *Polyglucoside granules.* Small, about 15 nm, electron dense granular structures are numerous in *T. thiebautii,* and located between the thylakoids in the cytoplasm. These granules are similar in appearance to polyglucoside granules (α-granules) which are known to store carbon and energy in form of carbohydrate. Carbohydrates have been proposed to function in balasting the cells and play a role in buoyancy regulation (Walsby 1972, Villareal and Carpenter 1990).

3.2.13. *Nucleoplasm.* In most cyanobacteria the nuclear material is centrally located in the cells. It appears as such in *T. erythraeum* cells (van Baalen and Brown 1969), while it seems to be scattered in *T. thiebautii* cells, as demonstrated in Fig. 27 using diamidino-phenylindole (DAPI) staining. The distribution of DNA in *T. thiebautii* resembles that reported for *Prochloron* (Swift 1990).

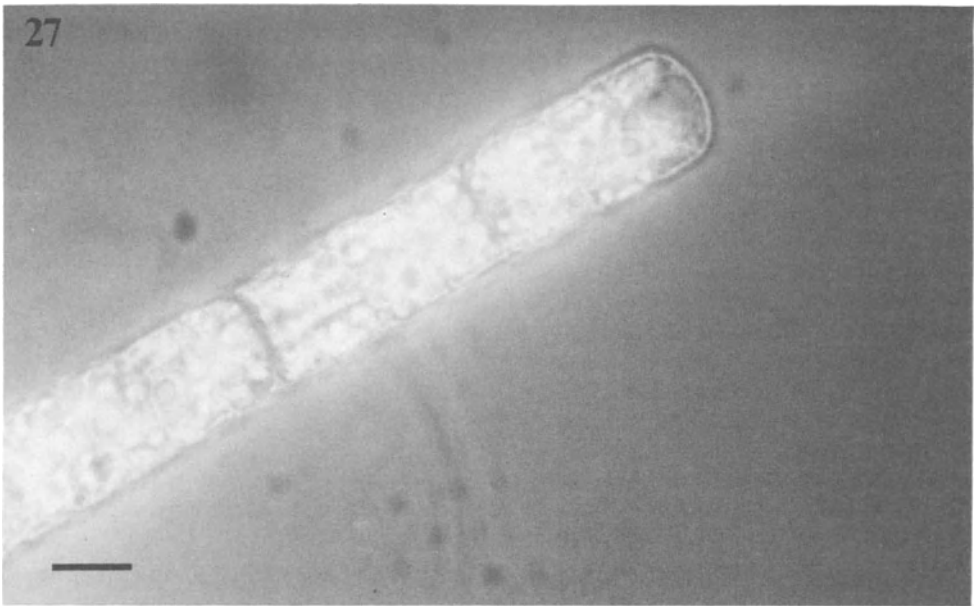


Figure 27. Epifluorescence micrograph of a *T. thiebautii* trichome stained with DAPI. The fluorescence indicates the scattered distribution of discrete clusters of the DNA material in the cytoplasm throughout the cells. Bar = 10 μm.

3.3. MORPHOLOGICAL AND ULTRASTRUCTURAL DIFFERENCES AMONG *TRICHODESMIUM* SPECIES

The ultrastructural organization of *Trichodesmium* cells in many respects is uniquely different from other cyanobacteria. Morphological characteristics and the presence, orientation and arrangement of subcellular inclusions appear to be species specific and may be employed for distinguishing *Trichodesmium* species. Key features characterizing differences between *T. erythaeum* and *T. thiebautii* have been detailed above and some are highlighted in Table 1. It should be kept in mind however, that studies on *T. erythraeum* have not been conducted in great detail and that the study of Haxo *et al.* (1987) was done on cultured material. An elaborate study on the morphology and fine structure of all marine species of *Trichodesmium* as well as characterization of the radial forms is indeed required and this may resolve the prevailing confusion in the usage of nomenclature leading to an ambiguity in the comparision of physiological data among species.

The apparent absence of cyanophycin granules, scroll-like bodies and vacuole-like structures together with the arrangement of gas vacuoles and DNA material in *T. erythraeum*, not only is in contrast in this respect to *T. thiebautii* at the ultrastructural level, but may also indicate possible differences in their functional biology. A detailed comparison of the ecological and physiological features of all *Trichodesmium* species would be interesting in unfolding both the external physical and chemical requirements and the intrinsic cellular regulation of their biological processes.

Table 1. Morphological and ultrastructural features characterizing *T. erythraeum* and *T. thiebautii.*

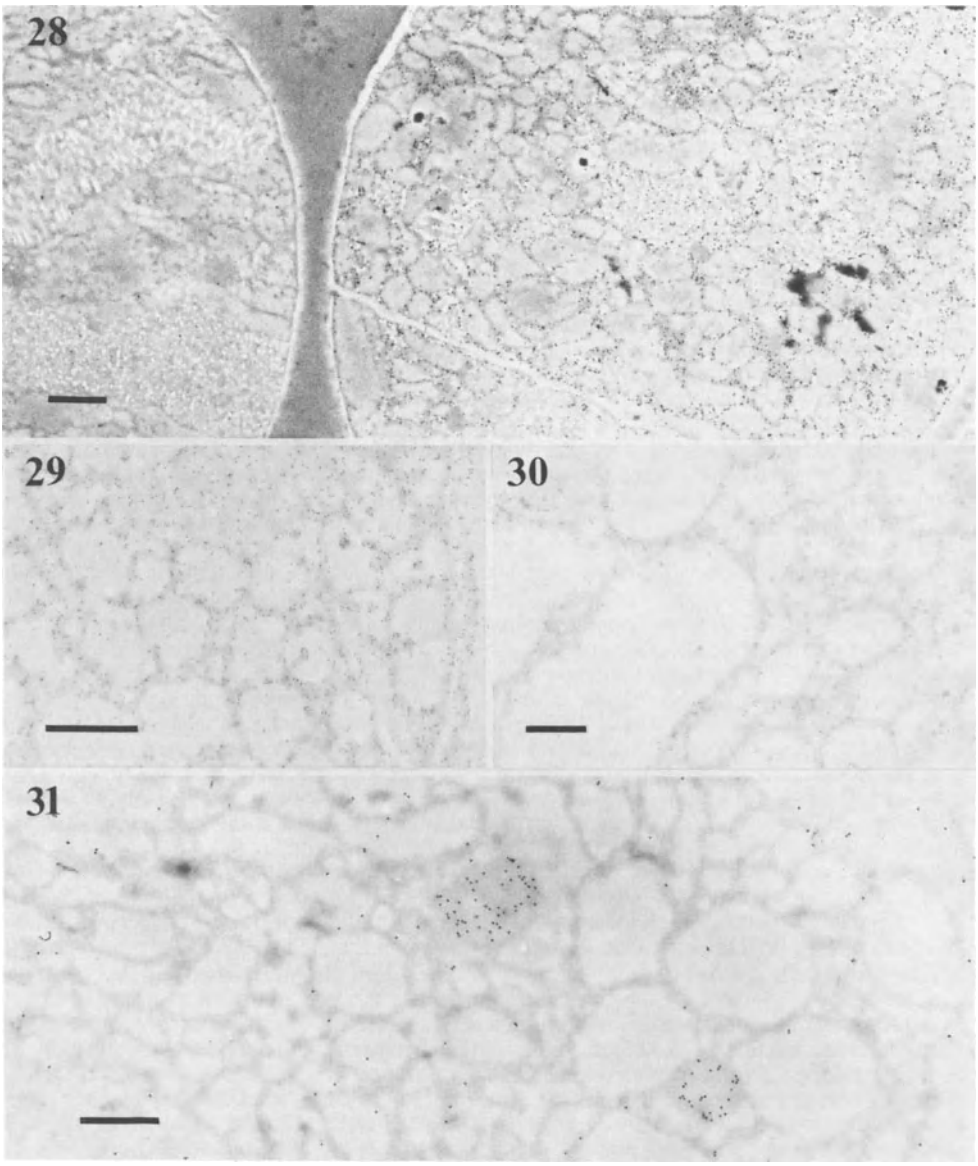
Characteristic Features	*Trichodesmium* Species	
	T. erythraeum	***T. thiebautii***
Colonial morphology		
Pigmentation	Dark, reddish-brown	Light, orange-brown
Trichomes	Straight and parallel	Slightly irregular & bending
Shape of colony	Fusiform, flat or raft-like	Fusiform, twisted like a rope, compact at the center and loose towards the edges
Cellular inclusions		
Vacuole-like structures	Not reported	Extensive
Gas vesicles	Arranged along the peripheral cell wall	Irregularly arranged
Polyhedral bodies	Present in the central core	Scattered
Cyanophycin	Not reported	Present in all cells except for the highly vacuolated cells.
Scroll bodies	Not reported	Present in all cells
Cylindrical bodies	1-3 per cell	Occasionally present
Polyphosphate bodies	Present	Absent
PHB-granules	Not reported	Frequent
Nuclear material	Centrally located	Scattered throughout the cell

3.4. DISTRIBUTION AND REGULATION OF KEY PROTEINS INVOLVED IN NITROGEN AND CARBON METABOLISM

3.4.1. *Nitrogen Fixation.* The earlier literature pertaining to nitrogen fixation and metabolism in *Trichodesmium* has been reviewed previously (Carpenter 1983, Fogg 1987). In *Trichodesmium*, the presence of nitrogen fixation and oxygenic photosynthesis is unique in that both the activities take place in the light in the apparent absence of cell differentiation. Studies have confirmed that *Trichodesmium* nitrogenase has a diel variation; its activity is initiated in the morning and peaks at about midday while there is no activity at night (Saino and Hattori 1978, Capone *et al.* 1990). Immunocytochemical localization of nitrogenase suggests *Trichodesmium* as the major nitrogen fixing member of the colony (Paerl *et al.* 1989, Bergman and Carpenter 1991). However, a number of additional bacteria (Maruyama *et al.* 1970, Currin *et al.* 1990) and the frequently occuring thin cyanobacterial filaments (Siddiqui *et al.* 1991b) have been shown to fix nitrogen in culture and to have nitrogenase, respectively. In *T. thiebautii* colonies collected from two widely separated areas (outside Bermuda and in the Sargasso Sea) nitrogenase containing trichomes were randomly distributed in the colony (Fig. 28) with a majority of the trichomes lacking the enzyme. Nitrogenase was not confined to the central core of the colony (Bergman and Carpenter 1991), as hypothesized earlier (Carpenter 1983). In some other *T. thiebautii* colonies, on the contrary, all the trichomes were seen carrying nitrogenase protein (Paerl *et al.* 1989). The reason for the discrepancy between these findings is not known, but the possibility exist, that all cells carry *nif* genes which however, may not be constitutively expressed in all trichomes. The fact that there may be a patchy induction of nitrogenase in a restricted number of trichomes and the variations in the proportion of *Trichodesmium* trichomes harbouring nitrogenase suggest that nitrogenase synthesis and activity may be a growth specific phenomenon (Siddiqui *et al.* 1991a) as is the case in the unicellular *Synechococcus* spp. (Leon et al. 1986, Mitsui *et al.* 1987). Alternatively colonies may be composed of two different *Trichodesmium* species, and only one of which can synthesize nitrogenase. Also our recent study on Rubisco and phycobiliproteins localization (see sections 3.2.2, 3.2.6 and 3.4.2), argues against the idea that the nitrogenase containing cells are functionally equivalent to heterocysts. Heterocysts in their mature nitrogen fixing stage lack carboxysomes (Braun-Howland *et al.* 1988, Rai *et al.* 1989, Renström-Kellner *et al.* 1990) which, on the contrary, is not apparently true for *Trichodesmium* (Siddiqui *et al* 1991a).

In cyanobacteria, fixed nitrogen, in the form of ammonia, is converted to glutamine and glutamate through a reaction catalysed by glutamine synthetase (GS) and glutamate synthase. Glutamine and in particular, glutamate are crucial constituents in the synthesis of amino acids, proteins, and nucleic acids. The nitrogen fixed in a *Trichodesmium* colony may either be used directly by the cell for growth or a part of it be excreted as, e.g. in symbiotic association (Rai 1990). As *Trichodesmium* colonies apparently have both nitrogen fixing and non-fixing cells (Bergman and Carpenter 1991), the non-fixing cells may fulfill their need for nitrogen by either taking up nitrogen released from the fixing cells or fixing nitrogen themselves; assuming that the non-N_2 fixing cells can induce nitrogenase at a different time (Bergman and Carpenter 1991). A detailed study of the fate of the fixed nitrogen and of the possibilities mentioned above in *Trichodesmium* is underway. Preliminary data indicate the presence of an active GS in *Trichodesmium* and that the protein is synthesized in all the cells both during the day and night. In addition, associated organisms may receive fixed nitrogen from *Trichodesmium* and thereby flourish within *Trichodesmium* colonies. The role, in this context, of some of the putative nitrogen fixing associated organisms (Currin *et al.* 1990, Siddiqui *et al.* 1991b) also requires further studies.

3.4.2. *Photosynthesis.* Phycoerythrin is a major light harvesting phycobiliprotein in *Trichodesmium* (Carpenter 1983). SDS-PAG electrophoresis and western blotting showed that PE in *T. thiebautii* is composed of α, β and γ subunits (Siddiqui *et al.* 1991a), a constitution similar to B- and R-phycoerythrin (Hoffmann *et al.* 1990). As given above (section 3.2.2), the immunocytochemical signal for both phycoerythrin and phycocyanin was observed in all cells (Siddiqui *et al.* 1991a) implying that phycoerythrin and phycocyanin are present in the nitrogen fixing cells as well. Carpenter and Price (1976) proposed that carbon fixation takes place only in the peripheral



Figures 28 - 31. Immunochemical localization and distribution of key proteins involved in the nitrogen and carbon assimilation in *T. thiebautii* . Fig. 28, part of cross-sectioned colony treated with anti (*Rhodospirillum rubrum*) nitrogenase antiserum and a 10 nm gold-IgG conjugate. Two cells from adjacent trichomes are seen, one with high levels of nitrogenase (right) and the other with a very low signal (left). Bar = 1 μm. Fig. 29, immunolocalization of phycoerythrin using anti (*Phormidium persicinum*) PE antiserum. Intense label (10nm gold) is seen in the vicinity of thylakoidal network. Bar = 5 μm. Fig. 30, immunolocalization of phycocyanin using anti (*Phormidium luridum*) PC antiserum. A less intense signal, but similar to PE in its localization, is seen. Bar = 0.5 μm. Fig. 31, part of cells immunized with anti (*Sinapis alba*) ribulose 1,5-bisphosphate carboxylase/oxygenase antiserum. The 10 nm gold spheres are preferentially localized in the polyhedral bodies and are also present in the cytoplasm. Bar = 0.5 μm.

parts of trichomes which pass through the colony center, and that the central regions of the colonies fix nitrogen. Similar observations relating peripheral granulated trichomes to carbon fixation have been made by Bryceson and Fay (1981). As mentioned above (section 3.2.6), the granulation of the trichomes was considered to be due to the presence of carboxysomes as opposed to the non-granulated cells lacking this inclusion. Recent observations indicate that polyhedral structures are present in all the cells throughout the *T. thiebautii* colony and that the polyhedral bodies contain Rubisco (Fig. 31) and are indeed carboxysomes (Li and Lee 1990, Siddiqui et al. 1991a). We have also evidence for the coexistence of Rubisco and nitrogenase within the same cells (Siddiqui *et al.* 1991a). Even though these findings disagree with the earlier proposed concept of spatial compartmentation of nitrogen fixation and oxygenic photosynthesis at least at the protein level, it does not exclude the possibility of a temporal separation of the two activities during the day time.

3.4.3. *Nitrogenase Protection.* The question of how *Trichodesmium* cells avoid oxygen inactivation of nitrogenase is highly interesting but still remains to be answered. Apparently, *Trichodesmium* does not seem to use the strategies of nitrogenase protection proposed for other cyanobacteria. Cell differentiation similar to that seen in the heterocystous species (leading to cell wall thickening, loss of PS II pigments and oxygen evolution, and loss of carboxysomes, etc.) does not exist in *Trichodesmium*, at least not at the ultrastructural and protein levels (Bergman and Carpenter 1991, Siddiqui *et al.* 1991a). Nor is there a temporal segregation of oxygenic photosynthesis and nitrogen fixation as in other non-heterocystous cyanobacteria (Carpenter 1983, Gallon and Chaplin 1988, Stal and Krumbein 1985). As opposed to the distribution pattern in *Trichodesmium* (Bergman and Carpenter 1990), high levels of nitrogenase was seen in all cells of non-heterocystous *Oscillatoria* sp. at peak nitrogenase activity in the dark (Stal and Bergman 1990).*Trichodesmium* apparently carries an oxygen-sensitive nitrogenase as implicated by the fact that under high oxygen atmosphere nitrogenase activity (acetylene reduction) declines drastically (D. G. Capone, personal communication), but its synthesis may be oxygen insensitive as is in the case of unicellular *Gloeothece* (Mullineaux *et al.* 1983). However, some recent findings, e.g. the high turnover rates of nitrogenase in *Oscillatoria* (Stal and Krumbein 1985) and the autoprotective mechanism of nitrogenase in *Azotobacter* (Thornely and Ashby 1989) are interesting mechanisms worth exploring in *Trichodesmium.* If photosynthetic oxygen production occurs simultaneously with nitrogen fixation, the protection of nitrogenase may be mediated through an efficient oxygen uptake machinery. This may include uptake of oxygen through respiration, uptake-hydrogenase, superoxide dismutase, or other possible means. Perhaps Rubisco functions as an oxygenase in *Trichodesmium* at midday (Siddiqui *et al.* 1991) when nitrogenase activity is at maximum (Saino and Hattori 1978, Capone et al. 1990), and carboxylase shows a distinct drop in activity (Li *et al.* 1980).

4. Concluding Remarks

A wide variety of organisms thriving within the *Trichodesmium* colonies render these buoyant aggregates as significant links in the productivity of nutrient poor oceans where *Trichodesmium* flourishes. Some species of bacteria and cyanobacteria growing in these colonies are probably capable of fixing nitrogen and thus, appear to share some of the physiological events taking place in *Trichodesmium* cells. Therefore, the presence of associated organisms should be given due consideration while planning experiments on *Trichodesmium* itself. This may be achieved by comparing data from natural populations and pure cultures. Also a comprehensive understanding of the significance of associated organisms and their interaction with *Trichodesmium* would be beneficial in this regard.

Recent studies on the presence, localization and activity of key proteins involved in carbon and nitrogen metabolism have altered the earlier concept of spatial segregation of carbon and nitrogen fixation in *Trichodesmium.* It also implies that the nitrogen fixing *Trichodesmium* trichomes do not differentiate cells into heterocysts. This, however, tends to complicate the understanding of nitrogenase protection from oxygen inactivation. Recently suggested mechanisms of high turnover rates of nitrogenase synthesis and the autoprotection of nitrogenase may be applicable to

Trichodesmium. A lowering of intracellular oxygen tension through a high activity of oxygen consuming enzymes, in combination with above mechanisms, may be important in maintaining the efficacy of nitrogenase activity.

5. Acknowledgements

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OXYGEN CYCLING IN CYANOBACTERIA WITH SPECIFIC REFERENCE TO OXYGEN PROTECTION IN *TRICHODESMIUM* SPP.

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ABSTRACT. *Trichodesmium* uses an unidentified mechanism to protect nitrogenase from oxygen inactivation. This paper considers the role of various oxygen consuming reactions, including photorespiration, "dark" respiration (cytochrome aa_3 activity), and Mehler reaction, in lowering net oxygen evolution during photosynthesis. Considering our basic understanding of these reactions in cyanobacteria, Mehler reaction activity was hypothesized to play a significant role because of its potential for supplying ATP through pseudocyclic photophosphorylation and reducing oxygen during photosynthesis. Light-dependent oxygen uptake and evolution rates were measured on field-collected samples of *Trichodesmium thiebautii* using membrane inlet mass spectrometry. Oxygen evolution followed a typical saturation response as a function of short-term exposure to different light intensities. Oxygen consumption in the dark and the light was unusually high for a cyanobacterium. The dark respiration rate was approximately 30% of the maximum gross oxygen evolution rate. Oxygen uptake was also light dependent, increasing in proportion to light intensity from 50 to 400 $\mu E\ m^{-2}\ s^{-1}$, then remaining constant up to 1700 $\mu E\ m^{-2}\ s^{-1}$. The high respiration rate resulted in a light compensation point of 280 $\mu E\ m^{-2}\ s^{-1}$. Application of the photosynthetic electron transport inhibitor, DCMU, caused a rapid inhibition of both oxygen evolution and the light-dependent portion of the oxygen consumption flux, indicating that the light-dependent respiration was due to Mehler reaction activity. Oxygen reduction by Mehler reaction, as opposed to $NADP^+$ reduction, accounted for approximately 55% of the electron flow through the light reactions. These high oxygen cycling rates may explain the occasional observations of depleted oxygen zones within *Trichodesmium* colonies and may contribute to the oxygen protection of nitrogenase.

1. Introduction

One of the more perplexing issues in cyanobacterial research has been the oxygen protection

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mechanism of nitrogenase in *Trichodesmium* spp. The fact that N fixation is not separated from photosynthetic oxygen evolution either in time (day vs. night) or space (heterocysts vs. vegetative cells) as in other aerophilic N_2-fixing cyanobacteria (but see Gallon and Stal, this volume) means that nitrogenase must either be unusually insensitive to oxygen or protected by a uniquely effective mechanism. There is fairly good, but primarily indirect evidence that N fixation accompanies oxygenic photosynthesis within given cells of *Trichodesmium*, and that photosynthesis is required for N fixation. Thus, the opposing requirements of the cell for photosynthetic oxygen production and low oxygen tension at the site of N fixation seem incompatible, an idea supported by the fact that several strategies have evolved in other cyanobacteria to separate these processes (Walsby, 1986). A recent review and analysis by Carpenter et al. (1990) concluded that oxygen protection in *Trichodesmium* is at the cellular (i.e. physiological and/or biochemical) level rather than at the level of the aggregation state which was previously thought necessary for development of low oxygen zones. This contention is also supported by recent evidence demonstrating an N_2-fixing capacity of single trichomes (Ohki and Fujita, 1988) and a random distribution of nitrogenase throughout colonies (Bergman and Carpenter, 1991).

Several cellular mechanisms have been proposed, including oxygen reduction coupled to an uptake hydrogenase (Saino and Hattori, 1982), superoxide dismutase catalyzed decomposition of superoxide (O_2^-) (Carpenter et al., 1990), and cellular partitioning of photosynthetic membranes and nitrogenase (Ohki et al., 1991), but none of these have been fully substantiated. In this paper I take a new approach through an analysis of oxygen cycling associated with the photosynthetic and respiratory processes. The essence of this approach is that respiratory activity in the light can greatly reduce net oxygen evolution when photosynthesis is proceeding rapidly. I present a novel hypothesis regarding the regulation of photosynthesis and respiration during nitrogen fixation that leads to significantly reduced oxygen evolution. The hypothesis will be discussed in the context of various known capabilities and limitations of cyanobacteria and, in particular, *Trichodesmium*, and I will provide some preliminary supportive data.

2. Oxygen cycling

2.1 BACKGROUND

There are three important observations that set the stage for a discussion of oxygen cycling in *Trichodesmium*. First is the fact that the interior of some colonies may (but do not always) have subsaturating oxygen concentrations under low to moderate light intensities (Paerl and Bebout, 1988; Carpenter et al., 1990), signifying that the rate of oxygen consumption can significantly exceed oxygen evolution under what would ordinarily be normal photosynthetic conditions. Second, all cells within *Trichodesmium* colonies exhibit variable chlorophyll fluorescence emission indicating that they all have the potential for oxygenic photosynthesis (Carpenter et al., 1990). And lastly, cultures of free *Trichodesmium* trichomes are capable of N_2 fixation, but only when photosynthetic electron transport is active (Ohki and Fujita, 1988).

The later observation points out the dependence of N_2 fixation on photosynthesis. It also rules out the possibility that respiration of microorganisms associated with natural colonies (reviewed in Carpenter, 1983) is required to set up low O_2 concentrations for N_2 fixation. The work of Ohki, Fujita and colleagues on cultured *Trichodesmium* has been critical in demonstrating the N_2-fixing capacity of free trichomes and focussing our attention on cell, rather than colony, attributes that allow N_2 fixation. Independently, Carpenter et al. (1990) also concluded that cell physiology had to be the key to understanding oxygen protection in *Trichodesmium* based on a synthesis of work on field-collected colonies and reasonable theoretical arguments. Thus, an appropriate approach to the oxygen protection mechanism is through an understanding of the interaction of cellular photosynthesis and respiration and how those processes influence net oxygen evolution.

The degree to which photosynthetic oxygen evolution must be balanced by oxygen uptake will depend on the oxygen tolerance of nitrogenase. There is some indication that nitrogenase is less sensitive to oxygen in *Trichodesmium* than in other N_2-fixing cyanobacteria (Ohki and Fujita, 1988; Ohki et al., 1991). Ohki and Fujita (1988) determined the relationship between acetylene reduction and ambient oxygen concentration in cultured *Trichodesmium* trichomes. Maximum acetylene reduction rates occurred at approximately 50% of air-saturated oxygen concentrations and were reduced by 30% at air saturation and 50% at 0.3 pO_2 or an O_2 concentration 50% above saturation. Interestingly, inhibition of acetylene reduction at 0.3-0.5 pO_2 was at a constant level for up to 2 h, suggesting that nitrogenase is relatively stable at high oxygen concentrations. Ohki et al. (1991) hypothesized that O_2 causes deactivation of the Fe-protein of nitrogenase reducing the overall catalytic efficiency. In addition, they proposed that a reduction in the O_2 concentration causes reactivation without the requirement for de novo synthesis. If their model is correct, then nitrogenase activity is under dynamic regulation by the intracellular O_2 concentration and essentially follows the pO_2 response described by Ohki and Fujita (1988) when cells experience oxygen concentrations over a broad range. One of the important implications of this type of regulation is that *Trichodesmium* would be able to tolerate significant swings in oxygen concentration without irreversible damage to nitrogenase, at least for periods of a few hours. Furthermore, the negative correlation and short term stability of the acetylene reduction rate at different pO_2 within the range of 0.1 to 1.0 (Ohki and Fujita, 1988) means that a complete reversal of the oxygen flux to a net consumption during photosynthesis is not required for N_2 fixation, but any reduction in net oxygen evolution would be beneficial in terms of increasing the rate of N_2 fixation.

Generally, oxygen cycling in plants or algae refers to photosynthetic and respiratory oxygen metabolism that occurs in the light. Although there are a number of oxidases that are not strictly tied to either photosynthesis or respiration, they are less important bioenergetically and will not be considered. A general review of oxygen cycling in plants has been presented by Badger (1985), wherein details beyond the scope of this paper may be found. Oxygen cycling in the light is driven by photosynthetic oxygen evolution (Hill reaction) and three principal oxygen uptake processes; photorespiration, Mehler reaction, and a cytochrome oxidase reaction which is associated with the classical dark respiration pathway. All three of the uptake

processes are regulated directly by the photosynthetic apparatus in cyanobacteria. The following descriptions of the regulation of these processes are not intended to be complete but are focussed on cyanobacteria and the specific condition of *Trichodesmium*.

2.2 PHOTORESPIRATION

Oxygen uptake by photorespiration is due to the oxygenase activity of ribulose bisphosphate carboxylase-oxygenase (Rubisco), the enzyme also responsible for the initial carboxylation step of the photosynthetic carbon reduction (PCR) cycle. In C_3 marine plants and some microalgae, photorespiration represents a substantial loss of fixed carbon and consumption of oxygen (Lloyd et al., 1977; Burris, 1981; Beardall, 1989). However, many microalgae and possibly all cyanobacteria possess a dissolved inorganic carbon concentrating mechanism that increases the *in vivo* concentration of CO_2 relative to O_2, thus pushing the competitive balance of the Rubisco reaction in the direction of carboxylation (Raven, 1985; Beardall, 1985, 1989; Aizawa and Miyachi, 1986). The fact that the photorespiratory pathway exists in cyanobacteria, however indicates that the oxygenase activity is not completely or at all times suppressed (Harris, 1980). Renström and Bergman (1989) found that there were differences among cyanobacteria species in the excretion of glycolate, indicative of photorespiration, when grown under normal CO_2 levels. This may reflect species differences in the effectiveness of the dissolved inorganic carbon (DIC) concentrating mechanism.

Very little work has been done on photorespiration of marine cyanobacteria and it is not possible to make general conclusions. Li et al. (1980) observed lower C fixation rates in *Trichodesmium* under high light (2100 μE m^{-2} s^{-1}) and oxygen concentrations of 20 and 84%. (The oxygen effect was not observed in incubations at 147 or 420 μE m^{-2} s^{-1}.) They suggested that the oxygen effect was due to photorespiration. Glover and Morris (1981) provided evidence of photorespiration in two *Synechococcus* strains, perhaps reflecting the lack of a DIC concentrating mechanism as Karagouni et al. (1990) recently suggested. This, however, needs verifying because Badger and colleagues (Badger and Andrews, 1982; Badger et al., 1985) rigorously demonstrated the existence of a concentrating mechanism in a different marine *Synechococcus* strain. More direct studies of the photorespiratory potential of marine cyanobacteria need to be done before we understand the magnitude of this process under natural conditions.

2.3 DARK RESPIRATION

Dark respiration is generally a small fraction of the energy budget of cyanobacteria although it is essential for supplying metabolic energy during normal dark periods. In general, cyanobacteria produce ATP by photophosphorylation in the light and by oxidative phosphorylation in the dark with the latter driven by carbohydrate reserves produced by photosynthesis. The effect of light on dark respiration is very different in cyanobacteria compared to eukaryotes. This is due to the limited cellular compartmentation in cyanobacteria and the apparent necessity for sharing certain electron transport components between photosynthetic and

respiratory pathways. Recent reviews on the regulation of electron transport between these pathways may be found in Matthijs and Lubberding (1988) and Scherer (1990).

It has been known for some time that oxygen uptake by cyanobacteria is reduced upon going from darkness to light at low to moderate intensities (Hoch et al., 1963). We now know that this is due to the competitive interaction of PS I and cyt aa_3 for electrons. Electrons coming from either PS II or NADH (via respiration) pass through the plastoquinone pool and the thylakoid-bound cyt b_6/f complex on their way to their respective terminal acceptors. In darkness, electrons donated to plastocyanin (or a soluble cyt c) are shuttled to cyt aa_3 because PSI is fully reduced in darkness. Upon illumination, PSI becomes oxidized and can compete for electrons. Scherer (1990) suggested that PS I outcompetes cyt aa_3 by virtue of a significantly greater turnover rate rather than differences in the affinity for electrons.

This scenario implies that oxygen uptake via dark respiration is essentially turned off in the light. However, this may not always be the case as there appears to be considerable flexibility in the presence and subcellular distribution of critical redox components of the respiratory chain. Localization studies have shown that some of the respiratory cytochromes (e.g. cyt aa_3) are located on the cell membrane (see Matthijs and Lubberding, 1988). Moreover, it has recently been discovered that a complete respiratory chain, including a cyt b_6/f-type complex, exists in the cytoplasmic membrane (Kraushaar et al. 1990) of some cyanobacteria. This means that respiratory and photosynthetic redox pathways may operate independently and that "dark" respiration may not always be turned off in the light.

2.4 MEHLER REACTION

The Mehler reaction is the reduction of oxygen by photosystem I with the formation of hydrogen peroxide. The initial step of the Mehler reaction is a one electron donation to molecular oxygen by either ferredoxin (or flavodoxin) or ferredoxin-NADP oxidoreductase, which have a tendency to autooxidize. The product of the reaction, superoxide (O_2^-), is converted to hydrogen peroxide (H_2O_2) by superoxide dismutase. The fate of hydrogen peroxide will be discussed below. The first demonstration of a physiological function of the Mehler reaction was recently made by Miller et al. (1988). They showed that the ATP required for active transport of DIC in *Synechococcus* UTEX 625 was produced by oxygen photoreduction and pseudocyclic photophosphorylation. This "priming" mechanism is probably the same as that described by Radmer and Kok (1976) for *Anacystis nidulans* in which oxygen photoreduction was pronounced during the first ca. 30 seconds after a dark to light transition. That is the period during which the DIC pump becomes active.

Robinson (1988) suggested that the Mehler reaction is a spill-over reaction for electrons in the photosynthetic electron transport chain when conditions cause the normal electron acceptor ($NADP^+$) to be fully reduced. Another way of viewing this is that $NADP^+$ is a better competitor than O_2 for electrons coming off PS I. This is probably due, at least in part, to ferredoxin being more efficient at transferring electrons to $NADP^+$ compared to O_2 as was

shown in chloroplasts (Furbank and Badger, 1983). This general regulatory scheme is consistent with the idea that oxygen reduction becomes important when the rate of NADPH turnover is low relative to the ATP demand, as during the induction phase of the PCR cycle.

CO_2 fixation at light saturation is another physiological condition that promotes Mehler reaction activity. At light saturation, the turnover of NADPH is limited by the speed of the PCR cycle. In theory, if the light intensity is increased within the saturation range, it should be possible to increase the rate of electrons passing (non-cyclically) through PS I if there is an alternative electron acceptor - e.g. oxygen. We have observed this in marine *Synechococcus* as an increase in the oxygen uptake rate beginning at a light level that just saturates carbon fixation (Fig. 1; see also Hoch et al., 1963). This is consistent with previous studies showing that the capacity of the light reactions is greater than that required to solely support maximal carbon fixation rates (see Robinson, 1988). We do not yet know, however, whether this rapid

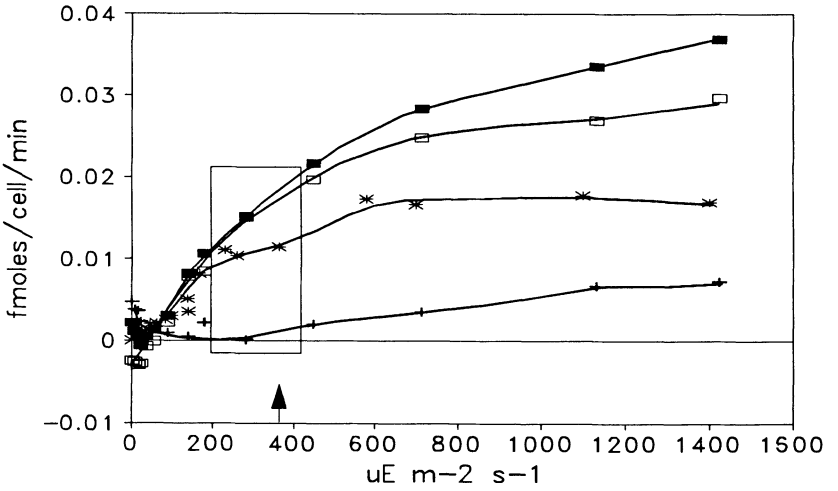

Figure 1. Effect of light intensity on oxygen uptake and evolution and ^{14}C assimilation in *Synechococcus* WH7803. Culture samples were adapted in exponential phase to 365 $\mu E\ m^{-2}\ s^{-1}$ (arrow) and analyzed for gross oxygen evolution (E_o: *closed squares*) and gross oxygen uptake (U_o: *pluses*) by mass spectrometry. Net oxygen exchange (*open boxes*) was calculated from the difference between E_o and U_o. Short-term (20-40 m) incubations with ^{14}C-bicarbonate were conducted in a parallel experiment using the same light source, to determine the carbon assimilation response (*asterisks*).

cycling of oxygen at high light intensities is coupled to photophosphorylation and is energy yielding, or uncoupled and a mechanism of energy dissipation under high light stress.

In the case of *Trichodesmium*, one can imagine that Mehler reaction activity can be used to modulate the supply of ATP and NADPH for N_2 fixation while at the same time contributing to a reduction in net oxygen evolution. When N_2 fixation occurs along with carbon fixation, the steady-state rate of ATP utilization relative to NADPH utilization is higher than under non-N_2-fixing conditions due to the relatively greater ATP demand of N_2 fixation compared to C fixation. Pseudocyclic photophosphorylation may be the source of this additional ATP in the same way it supports the DIC pump in *Synechococcus* UTEX 625. In tropical and subtropical waters where *Trichodesmium* occurs, it seems a distinct advantage, bioenergetically, to couple oxygen consumption with light-driven phosphorylation rather than oxidative phosphorylation. That way, light, rather than the oxidation of fixed carbon provides the energy for ATP production.

What is the extent to which oxygen evolution can be balanced by oxygen uptake via the Mehler reaction? The extreme situation is when all of the electrons derived from water are passed along to O_2. The normal stoichiometry under these conditions is 1 oxygen molecule evolved to 1 oxygen molecule reduced back to the level of water. Thus, there is no net change in oxygen concentration. As described above, the product of the Mehler reaction is H_2O_2, but it is necessary for cells to get rid of H_2O_2 because of the potential oxidative damage it can cause to lipids and proteins. Under normal physiological conditions, H_2O_2 is metabolized in cyanobacteria in one of two possible pathways, either through catalase or through an ascorbate peroxidase. Catalase causes the disproportionation of 2 H_2O_2 molecules to 2 H_2O and O_2. The ascorbate peroxidase reaction reduces H_2O_2 to 2 H_2O. Asada and colleagues have looked at these pathways in depth in cyanobacterial and eukaryotic sources. By determining the fate of $H_2{}^{18}O_2$ in illuminated cells, Miyake et al. (1991) were able to separate species that strictly use catalase (which produces $^{18}O_2$) from those that also have ascorbate peroxidase (which also produce $^{16}O_2$). The ascorbate peroxidase mechanism was light-dependent and associated with photosynthetic oxygen evolution, indicating that reductant for the reaction comes from the light reactions. Thus, even though H_2O_2 is completely reduced to H_2O, formation of reducing power requires the evolution of 0.5 O_2 from PS II.

It is possible to drive electrons completely to O_2 when carbon fixation is stopped either by CO_2 limitation or PCR cycle inhibitors. Miller et al. (1988) reported rates of electron transport at 70% of maximum rates when the PCR cycle was inhibited in *Synechococcus* UTEX 625 and during active transport of DIC. This was manifested as a one-for-one cycling of oxygen. It is intriguing to speculate that *Trichodesmium* cells on the inside of colonies may use the Mehler reaction to support N_2 fixation where CO_2 fixation is severely limited as Carpenter and Price (1976) have shown.

3. Methodology

Oxygen cycling was measured in *Trichodesmium* during an R/V Iselin cruise in waters west of the Bahama Islands and islands of the Turks and Caicos. *Trichodesmium* was collected by net tow at a depth of 25 m and isolated by hand-picking colonies and suspending in filtered seawater. All experiments were conducted with freshly collected samples and were generally performed within a 2 h period, end-to-end. *Trichodesmium* was analyzed as disrupted colonies to remove the effect of colony structure on the transport of CO_2 and O_2. There was no discernable effect of sample handling on photosynthetic rates during the experimental periods.

Oxygen cycling was measured using the membrane inlet mass spectrometry technique (Hoch and Kok, 1963; Radmer and Ollinger, 1980). A concentrated *Trichodesmium* sample (estimated 0.3-0.5 mg chl/l) was bubbled with nitrogen to lower the oxygen concentration and then injected into a cuvette with a radially mounted semipermeable membrane (GE Silicone MEM-213) attached to a vacuum line of the mass spectrometer. A bubble of ^{18}O labelled oxygen (mass 36) was injected, and the sample was stirred (500 rpm) until the total oxygen concentration (mass 32 and mass 36) was close to the normal oxygen saturation level of air-equilibrated sea water. After removing all bubbles, oxygen uptake and evolution rates were measured at increasing light intensities using a slide projector and neutral density filters. Gross rates of oxygen uptake (U_o) and evolution (E_o) were calculated according to Radmer and Ollinger (1980) using blank corrections determined on a sample without *Trichodesmium*.

4. Results

Oxygen cycling was extremely high for a cyanobacterium under normal assay conditions. An example of an irradiance response of E_o, U_o, and net oxygen exchange in a mid-day sample of *Trichodesmium* is shown in Figure 2. This example was selected from a dozen such experiments and is generally representative of what was found throughout the natural diurnal period. Photosynthetic oxygen evolution exhibited a typical response with saturation occurring at about 600 $\mu E\ m^{-2}\ s^{-1}$, similar to that found by Lewis et al. (1988) for ^{14}C uptake with dispersed *Trichodesmium* trichomes originating from a surface bloom. Oxygen uptake (U_o) was substantial in darkness, being approximately 30% of the absolute magnitude of maximal gross photosynthesis. U_o was also significantly affected by light intensity. Starting at a relatively low light intensity (approx. 50 $\mu E\ m^{-2}\ s^{-1}$), U_o rose in proportion to light intensity up to approximately 600 $\mu E\ m^{-2}\ s^{-1}$ at which point the response saturated. The net effect of the gross uptake and evolution fluxes was a light compensation point that was extremely high (280 $\mu E\ m^{-2}\ s^{-1}$) and a maximal net evolution rate that was a small fraction (1/6th) of the gross rate.

The cause of the light-dependent uptake was investigated using the photosynthetic electron transport inhibitor, DCMU, which stops the Mehler reaction as abruptly as it stops oxygen evolution. DCMU caused an immediate reduction of U_o in the light to a level slightly below the uptake rate in darkness (Fig. 3). When the cells were subsequently darkened, U_o

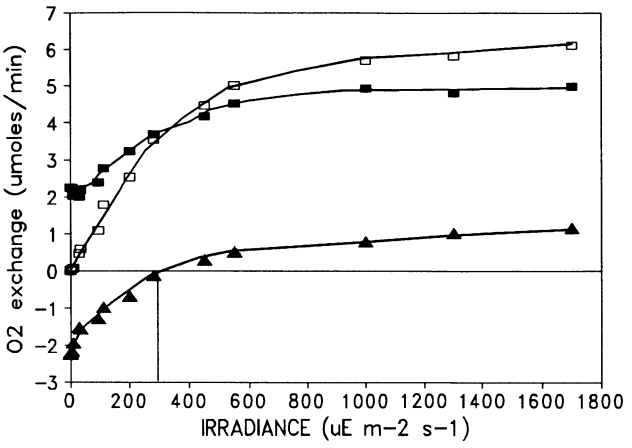

Figure 2. Effect of light intensity on gross oxygen evolution (E_o: *open squares*) and uptake (U_o: *closed squares*) and calculated net oxygen exchange (*triangles*) for *Trichodesmium* collected from 25 m depth at 1300 h.

returned (rose) to the original dark rate (without DCMU). The lower rate of U_o under DCMU + light can be explained by light causing PS I oxidation and the movement of electrons primarily through the photosystem rather than cyt aa_3.

5. Discussion

The ability of *Trichodesmium* to consume oxygen in the light is extraordinary. This is attributable to at least two phenomena: a high basal rate of respiration that appears to be light-independent and a significant Mehler activity that is light-dependent. The high rate of dark respiration was almost completely insensitive to light as indicated by the marginal reduction in rate as light increased from 0-50 μE m^{-2} s^{-1}. This is consistent with the effect of DCMU on oxygen uptake in light versus darkness. DCMU + light caused only a 20% drop in oxygen uptake compared to that in darkness. This means that the oxidation of PS I by light only incompletely stopped oxygen uptake indicating that most of the dark respiratory pathway was independent of photosynthetic electron transport. Undoubtedly some of this dark respiration was due to bacteria and other heterotrophs that were transferred with the colonies, but there is no way of estimating what that fraction was. If heterotrophic organisms were responsible for most of the dark respiration, there must have been a substantial flux of organic material,

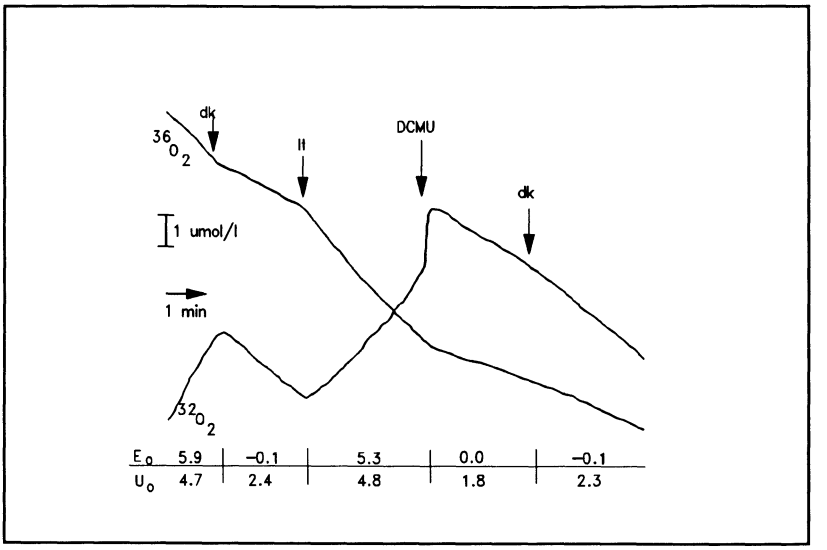

Figure 3. Tracings of uncorrected $^{32}O_2$ and $^{36}O_2$ concentration changes in a *Trichodesmium* sample subjected to saturating light, darkness, and DCMU. Mass 36 reflects oxygen uptake and mass 32 oxygen evolution and uptake. Sample began in the light (left) and followed (where indicated by arrows) by darkness, light, DCMU + light, and lastly DCMU + darkness. The significant jump in the mass 32 signal when DCMU was added was due to oxygen carried over with the inhibitor. Corrected gross evolution (E_o) and uptake (U_o) rates for each treatment are indicated at the bottom of the panel.

presumably from *Trichodesmium*, to drive it. Alternatively, if the dark respiration was due to *Trichodesmium*, then there must be a separate operational pathway for dark respiration. Future studies of the localization of electron transport components would be very enlightening.

Another interesting feature is the strong Mehler activity. The onset of Mehler reaction at relatively low light intensities was found to be a consistent feature in these types of experiments, suggesting that CO_2 fixation was saturated at low light. There are three possible reasons for this. First, it may reflect the light climate of the depth at which *Trichodesmium* was sampled, with the cells being relatively low-light adapted. In this case carbon assimilation would be saturated at a low light intensity. Self-shading within the bundled colonies would also tend to cause the average cell to be photoadapted to a lower than ambient light intensity. Second, the experimental conditions may have caused a lower CO_2 fixation rate due to a stress of some unknown nature. Although the criteria for healthy cells, i.e. no detectable reduction in

photosynthetic performance during the assay procedure, indicated that the chamber conditions were not stressful, disruption of colonies is known to alter N_2 fixation (acetylene reduction) (Carpenter and Price, 1976; Bryceson and Fay, 1981), suggesting that mechanical disruption has an effect on the physiological state of the cell. Lastly, the onset of Mehler activity at relatively low light intensity may reflect a greater demand for ATP than can be satisfied by normal photosynthetic electron transport. As described above, N_2 fixation would impose such a demand.

The fraction of electrons that flowed through the Mehler reaction as opposed to the normal NADP pathway can be determined from the ratio of the slope of U_o to the slope of E_o. That ratio ranged from 0.4-0.7 among all experiments with an average of 0.55. This means that 40-70% of the photosynthetic electrons are passed to oxygen rather than $NADP^+$. Although it is not known whether this pseudocyclic flow of electrons was coupled to ATP formation, there is no evidence that oxygen photoreduction can happen when ATPase is uncoupled (Badger, 1985). In fact, modulation of the ATP supply via feedback mechanisms seems to be determined by regulation of electron transport out of PS II (Weis and Lechtenberg, 1989). Therefore, it is likely that Mehler activity was responsible for approximately doubling the supply of ATP.

Oxygen uptake in the light had a pronounced effect on the net flux of oxygen. Net oxygen consumption at moderate irradiances is consistent with oxygen deficits measured within colonies in the light (Paerl and Bebout, 1988; Carpenter et al., 1990). The critical flux that causes such high light compensation points is the dark respiration rate. Mehler reaction alone would only cause a proportional reduction in the E_o rate and the light compensation point would be 0 μE m^{-2} s^{-1}.

It is clear that oxygen cycling can reduce the net amount of oxygen evolved during photosynthesis while maintaining an appreciable rate of photosynthetic electron transport. A large amount of work needs to be done to establish the actual significance of this oxygen cycling in terms of nitrogen fixation in *Trichodesmium*. What is the source of dark respiration? If it is from *Trichodesmium*, how do the cells regulate dark respiration when photosynthesis occurs? Is there unusual partitioning of cytochromes in *Trichodesmium*? Is Mehler reaction activity regulated by the demand for ATP? Does *Trichodesmium* have an unusually high capacity for detoxifying O_2^- and H_2O_2 produced by the Mehler reaction?

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Oxygen Dynamics in *Trichodesmium* spp. Aggregates

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ABSTRACT. Oxygen concentrations measured within, and surrounding aggregates of the planktonic diazotrophic cyanobacterium *Trichodesmium* spp. are the product of the balance of the relative rates of photosynthetic O_2 production and a number of O_2 consuming mechanisms. These O_2 consuming mechanisms may be grouped into those operating at: 1) the physiological level of the *Trichodesmium* trichomes themselves (physiological mechanisms), 2) the level of the hydrodynamical and diffusive effects of aggregate size and shape (physical mechanisms), and 3) the level of the O_2 budget of the entire community of organisms (including microheterotrophs) that occur within these aggregates (community mechanisms). Changes in the balance of production of O_2 from photosynthesis and removal of oxygen from these mechanisms result in aggregate internal oxygen concentrations (under conditions of saturating irradiance) which range from anaerobic to over 500 μM O_2 . Irradiance at sub-saturating levels would push the oxygen balance towards net O_2 consumption by the aggregates whereas reduced O_2 consumption would result in net O_2 evolution by the aggregates. Net oxygen evolution by aggregates is possible throughout the diel cycle, and some diel changes in the net oxygen flux from the aggregates are seen. Large differences are observed in the O_2 flux from aggregates of *T. thiebautii* and *T. erythraeum* at the same time of day, the former being a greater net producer of O_2. The observed individuality of O_2 dynamics suggests that photosynthetic performance, and O_2 consumption vary appreciably among aggregates on population and community levels. The observed differences in O_2 dynamics may be related to the relative diazotrophic cability of individual aggregates.

1. Introduction: The Paradox of Contemporaneous O_2 Production in Non-heterocystous Diazotrophic *Trichodesmium* Aggregates

Aggregates of the non-heterocystous planktonic cyanobacterium *Trichodesmium* spp. have been cited as being important contributors of fixed nitrogen to the oligotrophic regions of the ocean in which they occur (Carpenter, 1983). Diazotrophy (biological nitrogen fixation) in *Tricho-*

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desmium spp., in contrast with all other non-heterocystous cyanobacteria (heterocyst = specialized cell, found in many species of cyanobacteria, used to protect nitrogenase from O_2) appears to be confined to daylight periods (Saino and Hattori, 1978; Capone *et al.*, 1990), when photosynthetic O_2 production should inhibit the obligately anaerobic process (Yates, 1977) of diazotrophy. The ability of *Trichodesmium* to contemporaneously reduce both N_2 and CO_2 does not seem to rely on biochemically-unique photosynthetic properties or capabilities (either light or dark reaction) (Stewart, 1975). In pursuit of a mechanistic explanation for this unique capability, Fogg (1974) proposed that aggregation of *Trichodesmium* spp. could function to spatially localize (and hence separate) the O_2 producing process of photosynthesis from the O_2 sensitive process of diazotrophy. According to this hypothesis, photosynthesis would be most active in high light peripheral regions, while diazotrophy would be localized in the interior (possibly self shaded) regions of the aggregates. *Trichodesmium* is frequently reported as aggregates of either parallel- or radially-oriented trichomes, commonly referred to as "tufts" and "puffs" respectively. Characteristically, aggregates reveal a densely packed (in the case of puffs), or tightly wound (in the case of tufts), darkly colored (brown-black) central core region.

The apparent incongruity of O_2-production in close association with the anaerobic process of diazotrophy (both of which occur at high rates in illuminated *Trichodesmium* spp. aggregates) has attracted a number of efforts to characterize the oxygen environment within aggregates of *Trichodesmium* spp. over a number of years. Elaborating on Fogg's suggestion of spatial separation of the N_2 and CO_2 fixing processes, Carpenter and Price (1976) localized photosynthetic $^{14}CO_2$ incorporation among individual trichomes using microautoradiography. They noted spatial partitioning of CO_2 incorporation during short-term incubations. Portions of trichomes near the center of aggregates were found to contain less ^{14}C label than the portions of the trichomes near the ends of the aggregates. In complimentary studies, Bryceson and Fay (1981) and Paerl and Bland (1982) investigated cellular reduction of the low redox potential tetrazolium salt 2,3,5 triphenyl tetrazolium chloride (TTC) in intact diazotrophic *Trichodesmium* aggregates obtained from the Indian Ocean (*T. erythraeum*) and Western Atlantic (Gulf Stream) Ocean (*T. thiebautii*) respectively. In both studies it was noted that more intense and rapid TTC reduction took place among internal (as opposed to peripheral) regions of puffs and tufts. In neither study was it determined what proportion of the total population of aggregates exhibited these TTC reduction patterns.

A separation of the processes of diazotrophy and photosynthetic O_2 production between trichomes in a single aggregate (rather than between distal and proximal regions of individual trichomes) might also explain the unique ability of these aggregates to carry out both processes simultaneously. Bergman *et al.* (1991) have suggested that due to exclusive localization of nitrogenase in certain trichomes, these trichomes might be more active than others as sites of diazotrophy. However, the key CO_2 fixation enzyme 1,5 ribulose diphosphate carboxylase (Rubisco) appears to be present in trichomes located throughout the aggregates (Bergman, this volume). In an earlier study Paerl *et al.* (1989) found nitrogenase-positive cells to be more widely dispersed throughout *Trichodesmium* colonies. To what extent these different observations may reflect individuality among specific populations or communities or spatial partitioning of diazotrophy in relation to photosynthetic O_2 evolution, remains unknown. It must be cautioned, however, that the localization (and inference thereof of differential activity) of nitrogenase does not necessarily confer active diazotrophy, since immunopositive nitrogenase may be inactive due to O_2 inhibition. In addition, a culture of *Trichodesmium* spp., in which the trichomes do not form aggregates, fixes nitrogen at relatively high rates (Ohki *et al.*, 1986; Ohki and Fujita, 1988, 1991), raising the intriguing possibility of simultaneous CO_2 and N_2 fixation in a single trichome.

Further work (possibly autoradiography, and immunochemical localization of nitrogenase and Rubisco) should be directed towards clarifying whether individual trichomes in this culture do carry on both processes simultaneously or whether there is a partitioning between trichomes.

The development of O_2 microelectrodes with tip diameters of less than 10 μm across (reviewed in Revsbech and Jorgensen, 1986) has made possible direct measurements of O_2 concentrations within freshly collected aggregates of *Trichodesmium* spp. (Paerl and Bebout, 1988; Paerl *et al.*, 1989, Carpenter *et al.*, 1990). These measurements reveal that the aggregate internal O_2 concentrations are highly dynamic, and range in concentration from 0 to over 500 μM. Under conditions of saturating irradiance, both highly oxic and anaerobic internal regions have been reported (Paerl and Bebout, 1988; Carpenter *et al.*, 1990). These conflicting measurements are an obvious source of confusion for the investigator interested in defining the environment faced by nitrogen fixing trichomes of *Trichodesmium* spp. However, since aggregates of *Trichodesmium* spp. are a complex association of trichomes of *Trichodesmium*, as well as bacteria, other cyanobacteria, diatoms, and arthropod and protozoan grazers, the O_2 concentrations present within the aggregates are the result of a number of tightly coupled and highly dynamic O_2-producing and O_2-consuming processes. In this contribution we will discuss our measurements of O_2 concentrations within aggregates of *Trichodesmium* spp. and attempt to describe some of the factors responsible for these O_2 concentrations.

2. Materials and Methods

2.1. OXYGEN MICROELECTRODE MEASUREMENTS

The techniques used to make O_2 microelectrode measurements within aggregates of *Trichodesmium* spp. have been described in detail elsewhere (Paerl and Bebout, 1988; Paerl *et al.*, 1989) but will be briefly summarized here. We used electrodes of both the cathode and micro-Clark types (Revsbech and Jorgensen, 1986). Electrode current was read (and polarization voltage supplied by) a Diamond General Chemical Microsensor (model 1201). *Trichodesmium* aggregates were collected by plankton net in most cases (diver collected and bucket collected aggregates were also examined). Aggregates were placed in a shallow dish for microelectrode measurements and held in place with a few teased-up fibers of a glass fiber filter attached to the dish. Temperature control was obtained with a water bath surrounding the specimen dish. Electrode placement was controlled with a micromanipulator, and was observed under a dissecting microscope. The specimen holder, microscope, and micromanipulator were mounted on a gimbal table in order to isolate them from the motion of the ship. Electrodes were calibrated using nitrogen and air bubbled seawater standards. Illumination was provided through a fiber optic light source equipped with a with a standard slide projector halogen lamp.

Concentration measurements were made within aggregates by advancing the electrode from the water surrounding the aggregate into the aggregate at 100 μm intervals. In addition to the concentration measurements, we made measurements of the change in O_2 concentration that occurred at a single point in the aggregate upon illumination. For these measurements, the electrode was generally positioned at the aggregate surface. In order to be able to compare measurements such as these for a number of aggregates, we took care to select very similar-sized aggregates of a given morphology. Net O_2 evolution for these experiments is expressed as net change in O_2 at the aggregate surface per period of illumination (generally 4 minutes).

2.2. $^{14}CO_2$ INCORPORATION BY AGGREGATES

Uptake of radiolabeled bicarbonate by aggregates was followed by incubating 10 aggregates in 10 ml seawater in glass serum bottles in an on-deck flow-through incubator. A 0.2 ml (1.4 μCi) aliquot of $^{14}C\text{-}HCO_3^-$ (specific activity = 58 μCi·mmole^{-1}; ICN Radiochemicals Inc.) was added to each bottle. Upon termination of a two hour incubation, the contents of the bottles were gently filtered onto glass fiber filters and rinsed twice with 0.2 μm filtered seawater (5ml per rinse). The filters were then fumed with concentrated HCl in order to remove inorganic carbon and the radioactivity on the filters was measured in a liquid scintillation counter. In order to determine aggregate-specific rates of uptake of $^{14}C\text{-}HCO_3^-$, individual aggregates were picked off the filter paper after HCl fuming and counted separately.

2.3. MICROAUTORADIOGRAPHY

Microautoradiographs were prepared from $^{14}CO_2$ incorporation assays. Isotope addition and incubation conditions were identical to those described above, except that both short-term (30 min.) and longer-term (2 h.) ^{14}C incorporation were examined. This was done by incubating an additional parallel set of serum bottles which was subsampled. At each incubation interval approximately 3 to 5 aggregates were fixed in 2% borate-buffered (pH 8.0) formalin and carefully transferred (by wide mouth Oxford macro-set pipette) to pre-wetted 25 mm diameter, 0.45 μm porosity HA Millipore filters. Aggregates were gravity filtered, followed by 2-5 ml pre-filtered seawater rinses. Filters were then rinsed with 0.01 M phosphate buffered saline to remove excess sea salts (which lead to fogging and subsequent interference with microscopic viewing), followed by air drying. On several occasions individual aggregates were frozen after formalin fixation. These aggregates were then bisected with a razor blade; efforts were made to section each aggregate through the central core region. Sectioned aggregates were then placed on filters as described above.

Air-dried Millipore filters were optically cleared and fastened onto cleaned microscope slides by passing the slides over fuming acetone (Paerl and Stull, 1979, Paerl *et al.*, 1989). Microautoradiographs were prepared using Kodak NTB-2 nuclear track emulsion diluted 1:1 with deionized water as described by Paerl *et al.* (1989). Exposure times ranged from 3 days to 2 weeks.

2.4. TETRAZOLIUM REDUCTION BY AGGREGATES

On selected occasions the cellular reduction of the low redox potential (E'_h) tetrazolium salt 2,3,5-triphenyl tetrazolium chloride (TTC) was microscopically observed. Reduction of this salt to its red insoluble formazan crystal has previously been show to be a good indicator of O_2-deplete microzones in which highly reduced processes such as N_2 fixation could be harbored (Fay and Kulasooriya, 1972; Bryceson and Fay, 1981; Paerl and Bland, 1982). In current work, TTC was added under illuminated and dark conditions to 10 ml serum bottles containing 5-10 *Trichodesmium* aggregates at a concentration of 0.01%. Samples were incubated for periods lasting from 15 min. to 2 h., after which they were fixed in 2% borate-buffered (pH 8.0) formalin. Individual aggregates were then viewed by phase contract microscopy and photographed.

3. Results

3.1. OXYGEN CONCENTRATION WITHIN AGGREGATES

Our measurements of O_2 concentrations within, and surrounding aggregates of *Trichodesmium* spp. have led us to few generalizations concerning the O_2 concentration within a "typical" aggregate. In addition to previously published microelectrode profiles of O_2 gradients within aggregates (Paerl and Bebout, 1988; Paerl *et al.*, 1989; Carpenter *et al.*, 1990), the profiles shown in Fig. 1 show that aggregates under conditions of saturating irradiance may contain regions of high or low O_2 concentration. Additionally, we were unable to detect differences in O_2 concentration between aggregates having the "tuft" or fusiform morphology and aggregates having the "puff" or radial morphology.

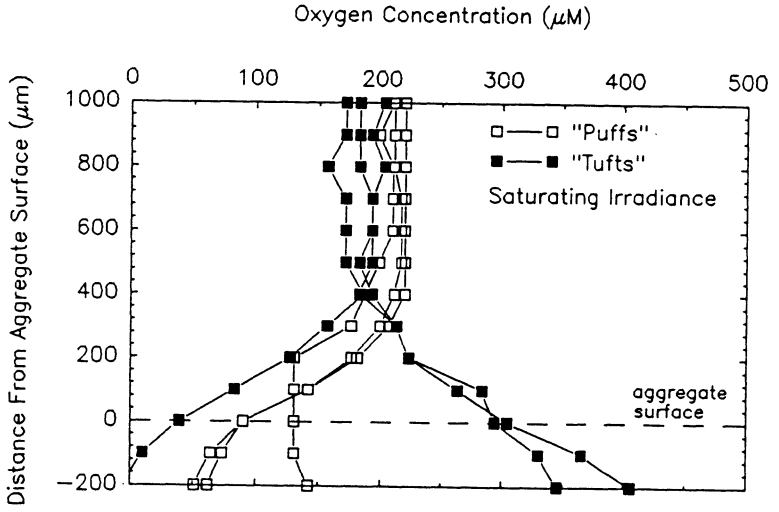

Figure 1. Oxygen concentrations within and surrounding aggregates of *Trichodesmium* spp. Profiles for both "tuft" and "puff" aggregate morphologies are shown. All measurements were made with freshly collected aggregates (under conditions of saturating irradiance) after aggregates had reached steady-state O_2 concentrations.

3.2. DIEL MEASUREMENTS OF NET O_2 PRODUCTION

Photosynthetic capability of aggregates of *Trichodesmium thiebautii* (expressed as net O_2 evolution over a 4 minute period of saturating irradiance) appears to be retained throughout the night as well as during the daytime. Some results from these types of measurements are shown for two diel periods in Fig. 2. With the exception of one peak in O_2 evolution by aggregates at 1800 hours and a trend towards reduced O_2 production in the late morning, net O_2 production by aggregates of *T. thiebautii* over the course of a diel period appears to exhibit little change (Fig. 2). This contrasts with measurements of diazotrophy made by Capone, et al. (1990) in which diazotrophy appeared to be possible only during the daytime.

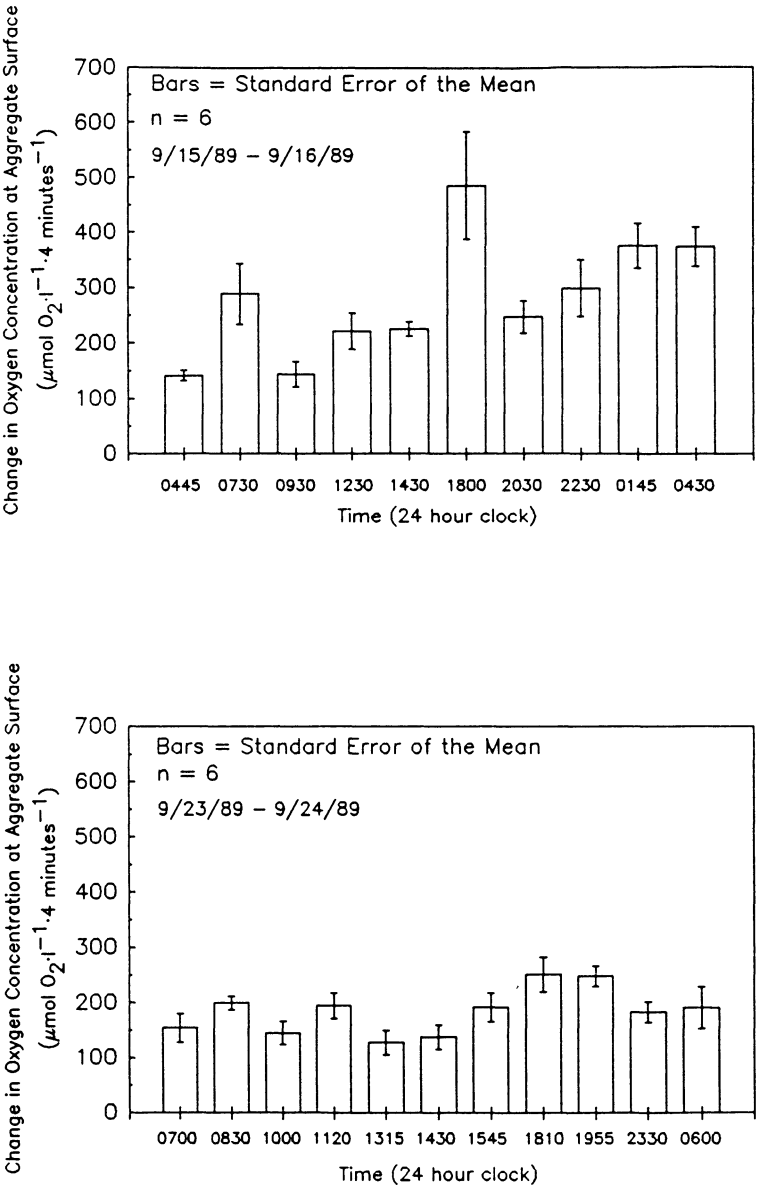

Figure 2. Diel patterns of net oxygen evolution by aggregates of *Trichodesmium thiebautii*. Measurements were made by placing the tip of an oxygen microelectrode at the surface of an aggregate and recording the change in oxygen concentration at that point over a four minute period of saturating illumination. Aggregates often did not reach steady-state. Measurements are shown for two diel periods.

3.3. NET O_2 EVOLUTION BY DIFFERENT SPECIES OF *TRICHODESMIUM*

We observed a strong difference in net O_2 evolution between two species of *Trichodesmium*. *T. erythraeum* was often observed to produce far less O_2 on a per colony basis than *T. thiebautii* (Fig. 3).

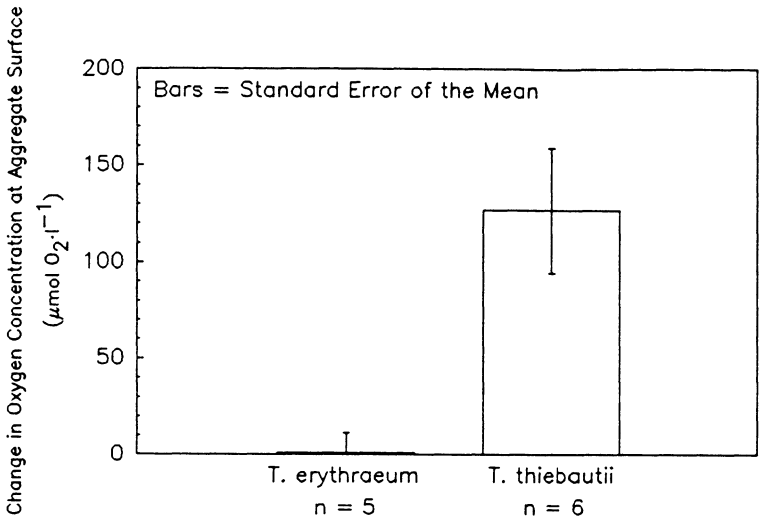

Figure 3. Net oxygen evolution by two species of *Trichodesmium*. Oxygen evolution was determined over a four minute period of saturating illumination at the surface of the aggregates. All aggregates were collected from the same net tow, and were similar in size.

3.4. CARBON FIXATION AND O_2 EVOLUTION ON A PER COLONY BASIS

Microautoradiographs of $^{14}CO_2$ fixation revealed a great deal of variation, both in cellular and regional incorporation. The existence of relatively low $^{14}CO_2$ fixing activities in internal regions of intact aggregates and partitioning of $^{14}CO_2$ fixation within individual trichomes, as first shown by Carpenter and Price (1976), were confirmed here. Rates of carbon fixation expressed on a per colony basis, proved highly variable (Table 1). Microautoradiography of individual aggregates revealed that there was a large degree of variability in the number of silver grains reduced by individual cells (an index of the cell specific incorporation of $^{14}C\text{-}HCO_3^-$, Fig. 4). Oxygen evolution, expressed on a per colony basis, was also highly variable. In addition, net O_2 evolution correlated poorly with with chlorophyll a concentration in the aggregates (Fig. 5).

3.5. THE EFFECT OF DISSOLVED O_2 CONCENTRATION ON AGGREGATE MORPHOLOGY

Manipulative experiments have given some indication that colony size and "tightness" can be altered by dissolved O_2 concentration. Within 1 h of doubling ambient O_2 concentration, formerly-dispersed aggregates revealed compaction or tightening accompanied by noticeable

Table 1. ^{14}C incorporation by individual aggregates of *Trichodesmium* spp. The means and standard deviations of the measurements are shown at the bottom of the table. Chlorophyll a concentrations for individual aggregates are also shown, but do not correlate with the aggregates used for ^{14}C measurements.

	"Tuft" Shaped Aggreggates		"Puff" Shaped Aggreggates	
	^{14}C Incorp. ($\mu g\ C\ h^{-1}$)	[chl a] (μg)	^{14}C Incorp. ($\mu g\ C\ h^{-1}$)	[chl a] (μg)
	0.080	0.049	0.018	0.034
	0.089	0.081	0.046	0.026
	0.065	0.018	0.083	0.061
	0.110	0.043	0.110	0.045
	0.129	0.035	0.080	0.032
	0.083	0.062	0.064	0.081
	0.054	0.066	0.064	0.022
	0.121	0.040	0.083	0.066
	0.097	0.022	0.075	0.047
	0.034	0.020	0.067	0.015
	0.107	0.042	0.024	0.027
	0.069	0.061	0.059	0.039
	0.046	0.019	---	---
	0.047	0.072	0.064	0.041
	0.071	0.029	0.026	0.020
	---	---		
Mean	0.080	0.044		
Std. Dev.	0.029	0.021		

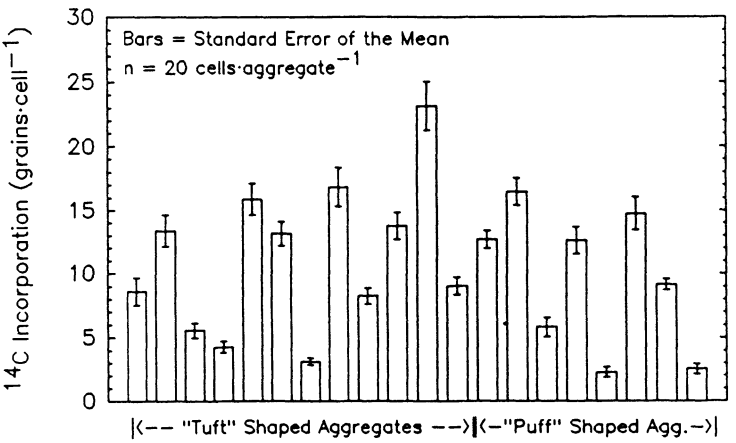

Figure 4. Cell specific incorporation of ^{14}C-labeled HCO_3^- by trichomes of *Trichodesmium* spp. Silver grains were counted in 20 haphazardly selected cells in each aggregate.

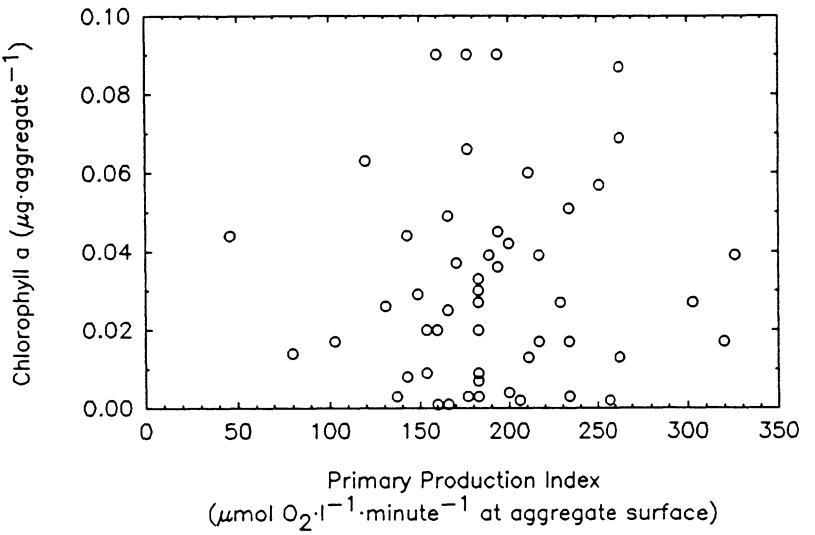

Figure 5. Net oxygen evolution by aggregates of *Trichodesmium* spp. and chlorophyll concentrations obtained for the same aggregates. Aggregates were collected over an entire diel cycle and were given a four minute period of saturating illumination.

darkening, presumably due to the densely packed trichomes in the internal core regions (Fig. 6). This response could be reversed (again within a period of approximately 1-2 hours) by lowering ambient O_2 tension (N_2 flushing) to saturated or subsaturated levels. These observations suggest an adaptive morphogenic change in response to oxygenation; tight aggregation (and presumably enhanced self-shading and reduced photosynthetic O_2 evolution of internal regions) as a response to the necessity to lower aggregate internal O_2 tension. This O_2-induced tightening response occurred under both illuminated and dark conditions. However, the maintenance of tight, compact conditions was more profound and persistent under continued illumination (and thus O_2 evolving conditions).

3.6. MICROAUTORADIOGRAPHIC AND TTC REDUCTION OBSERVATIONS

Observations presented here confirmed earlier microautoradiographic and tetrazolium reduction results indicating the presence of highly reduced (and hence low O_2 partial pressure) conditions coupled to either low or absent photosynthetic CO_2 fixing activities in internal regions (Fig. 7), even during periods of bright illumination. Additionally, these studies indicate that; 1) not all aggregates reveal internal reduced microzones, and 2) differences in cellular reduction and photosynthetic rates vary substantially within and among puff and tuft-shaped aggregates.

Using microautoradiography, it was demonstrated that aggregates exhibiting varying rates of photosynthetic production ($^{14}CO_2$ incorporation) and partitioning of ^{14}C labeling (initially confined to peripheral regions) were colonized by a diverse suite of microheterotrophs capable of incorporating a range of organic substrates (Paerl *et al.*, 1989; Fig. 8). In particular, amino acids and sugars were readily taken up and presumably respired (to an unknown extent) by these microbial epiphytes and consorts; thus representing localized (microscale) O_2 consuming activity.

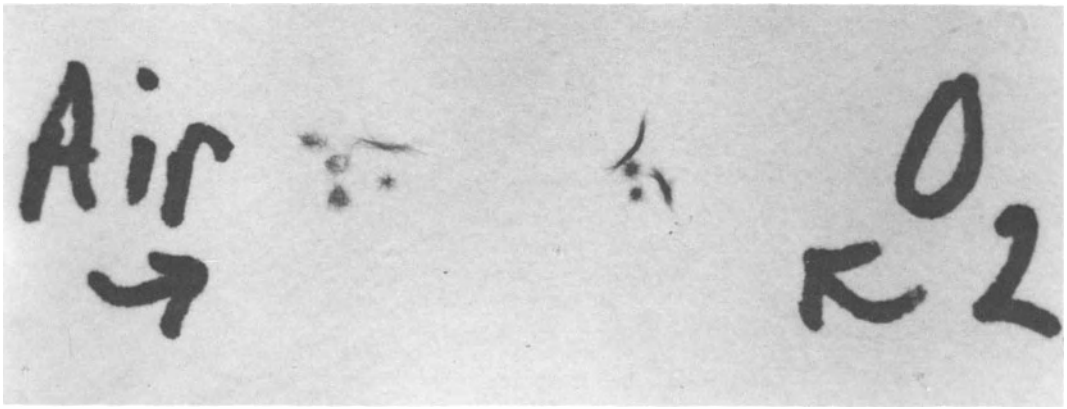

Figure 6. The effect of changing ambient oxygen concentration on aggregate morphology in *Trichodesmium* spp. Aggregates on the right were exposed to a one hour period of doubled ambient oxygen concentration.

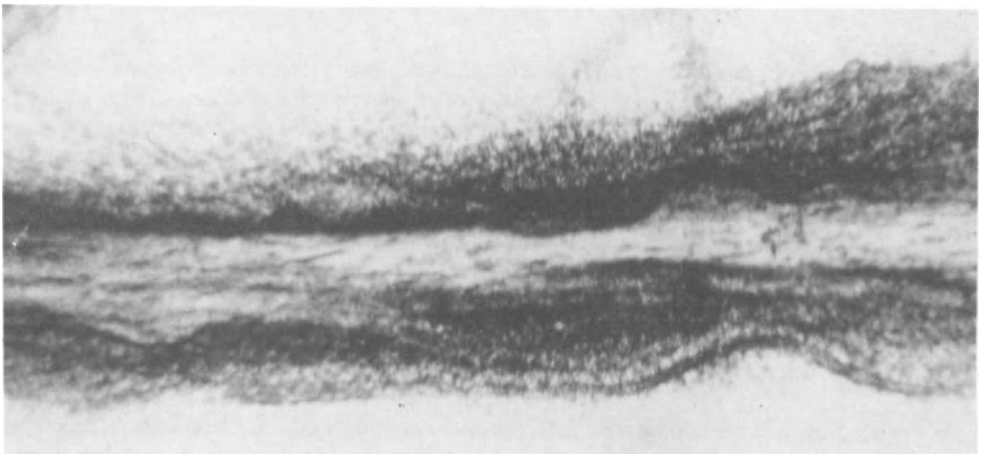

Figure 7. Microautoradiographic examination of $^{14}CO_2$ incorporation in a *Trichodesmium* spp. tuft after a 30 minute incubation under natural illumination (700 $\mu E \cdot m^{-2} \cdot s^{-1}$). The tuft was formalin-fixed and bisected prior to microautoradiographic exposure. ^{14}C incorporation can be seen as the deposition of opaque (black) silver grains in the overlying emulsion. Note the relatively low levels of ^{14}C incorporation in the internal regions of the aggregate.

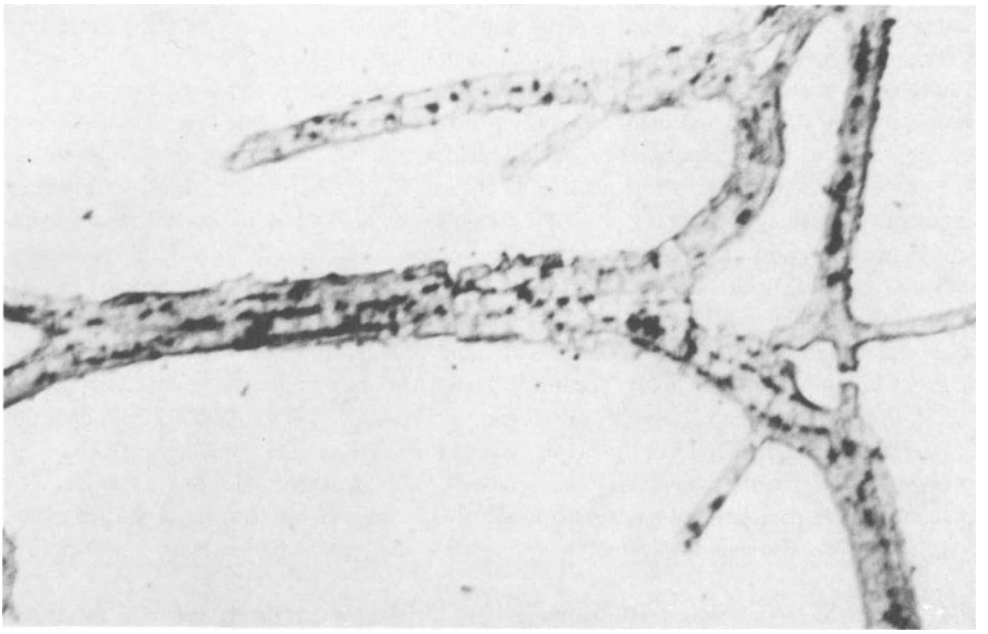

Figure 8. Incorporation of a ^{3}H-labeled amino acid mixture by microheterotrophs associated with *Trichodesmium* spp. trichomes. ^{3}H incorporation is seen as dense clusters of exposed silver grains overlying individual rod-shaped and filamentous microheterotrophs attached to trichomes in various locations.

4. Discussion

4.1. FACTORS AFFECTING O_2 CONCENTRATION WITHIN AGGREGATES OF *TRICHODESMIUM* SPP.

O_2 production within aggregates of *Trichodesmium* spp. results solely from the light dependent dissociation of O_2 from water molecules. O_2 consuming processes within the same aggregates include: 1) respiration (including photorespiration), of the cyanobacteria themselves as well as more specialized O_2 consuming mechanisms within the trichomes of the cyanobacteria, 2) the interaction of bundle size, shape, and degree of compaction with the diffusion of O_2 to and from the aggregate, and 3) respiration of the associated microheterotrophs and grazing organisms. Each of these O_2 consuming reactions is considered in greater detail elsewhere (Cunningham and Capone, this volume; Gallon, this volume; and Kana, this volume), and hence will only be briefly discussed below. The O_2 flux from (or to) a given aggregate, and the resultant internal O_2 concentrations within the aggregate, may be considered to be the product of the relative magnitude of the contribution of each of the above O_2 producing and O_2-consuming factors. Low rates of photosynthesis, or high rates of O_2 consumption, would push the aggregate towards net O_2-consumption, with resultant lower O_2 concentrations within the aggregate.

4.1.1. *Physiological Mechanisms*. *Trichodesmium* spp. (like other cyanobacteria, including other non-heterocystous cyanobacteria) possess a number of mechanisms which may lower intracellular O_2 concentrations under photosynthetic conditions. Mitochondrial respiration, photorespiration, and non-cyclic electron flow through PS I (Mehler reaction, Kana, this volume) all have the overall net effect of oxygen consumption. In addition, *Trichodesmium* spp. is reported to have superoxide dismutase activity (Cunningham and Capone, this volume) which, in combination with the superoxide-producing Mehler reaction, would lead to the theoretical capability of *Trichodesmium* to hold net O_2 flux at zero (Kana, this volume). Little information is available concerning the relative rates of these processes in *Trichodesmium* and clearly more work is necessary in order to evaluate the effectiveness of these cellular-level O_2 protective mechanisms. Work with a culture of *Trichodesmium* which is able to fix nitrogen and carbon simultaneously (Ohki *et al.*, 1986; Ohki and Fujita, 1988, 1991) certainly attests to the potential importance of these mechanisms. The presence and abundance of individual *Trichodesmium* trichomes in nature have been poorly documented relative to the vast amount of attention focused on diazotrophy and photosynthesis associated with aggregates. As a result, it has been difficult to ascertain whether individual trichomes form a detectable and significant fraction of *Trichodesmium* community biomass. The ability of individual trichomes to fix CO_2 and N_2 simultaneously in nature would obviate the need for O_2 protective mechanisms at the aggregate level, leaving us with a quite different interpretation of the reasons behind bundle formation.

4.1.2. *Physical Mechanisms*. *Trichodesmium* spp. trichomes frequently occur in nature as aggregates of trichomes. Therefore, in addition to physiological determinants of O_2 concentration, the contributions of physical factors such as aggregate size, and morphology, as well as hydrodynamical considerations such as intensity of the flow of water around the aggregate (as it moves through the water column) can affect the diffusive flux of O_2 from or to the aggregate. The shape of an aggregate has a large effect on the rates at which O_2 can enter and leave the aggregate. It has been shown in another aggregate forming cyanobacterium, *Aphanizomenon flos aquae*, that the individual trichomes within the aggregate are apparently able to coordinate movements which enhance the ability of the aggregate to expel photosynthetically-produced O_2 into the surrounding medium (Carlton and Paerl, 1989). Although it is not well documented, a number of anectdotal observations suggest that *Trichodesmium* spp. may also be able to change aggregate morphology, presumably to its advantage. Observations on individual aggregates spanning several hours reveal a complex interplay between shape and size. Tufts, particularly large tufts, can metamorphose into several small loosely organized puffs; the central region of the original tuft being pinched off, thus yielding two residual puffs. Those puffs eventually grow in size, and when fully formed exhibit a characteristic dense core region. Little information concerning the formation and metamorphosis of tufts has been obtained. Under conditions of enhanced turbulence (stirring, shaking and wave action) large tufts can be seen fragmenting into smaller ones, each slowly growing in size over time (H.W. Paerl, personal obs.).

Aggregate morphology is the product of the collective (and presumably coordinated) movement of individual filaments which can glide in parallel, converging, diverging, and at times perpendicular, fashion to each other. Among tightly-wound central regions of tufts and core regions of puffs (Fig. 9), individual trichomes are bound closely to each other. In contrast,

peripheral regions of both aggregate types appear to lack tight associations among trichomes, with noticeable inter-trichome spaces evident (Fig. 9). The degrees of "tightness" or "looseness", however, vary substantially over time, being frequently altered on time scales of minutes to hours. Plankton samples can yield both aggregate shapes or they can show high degrees of dominance by one morph. Aggregate shape and "tightness" would both play major roles in the rates of diffusion of O_2 to and from the aggregate. By altering the degree of

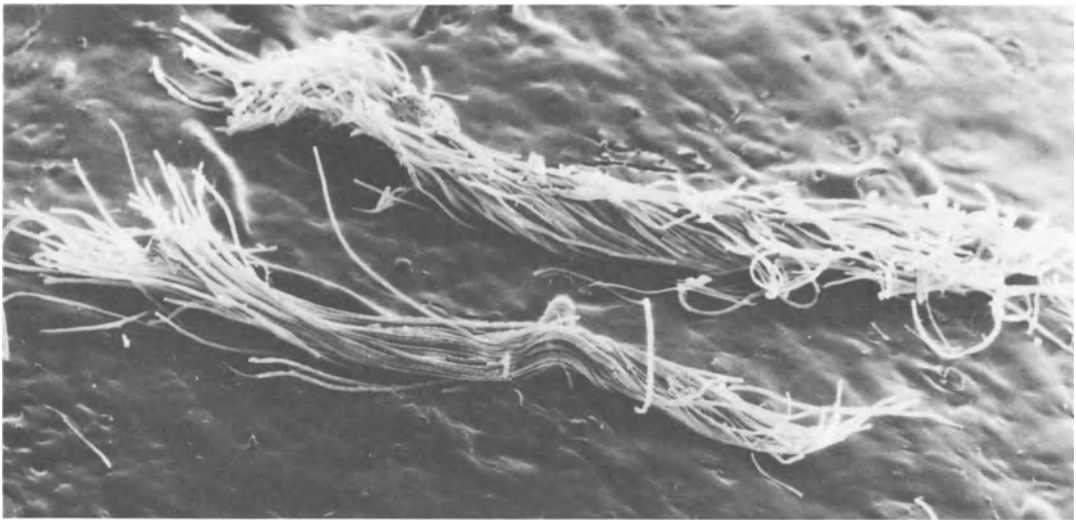

Figure 9. Scanning electron micrographs of aggregates of *Trichodesmium* spp. Two aggregates having the "tuft" morphology are shown.

compaction of the aggreggate, both the substance through which the O_2 diffuses (cellular material vs seawater), and the tortuosity of the diffusion path are altered.

In addition to modifying diffusion characteristics, aggregation also affects cellular self shading and light scattering, both of which in turn impact O_2 dynamics. While the degrees and magnitudes of self shading and light scattering, particularly in the densely-packed core regions of aggregates, are currently not established, their interactions with the rates of O_2 production from trichomes may play a key role as factors controlling aggregate O_2 dynamics.

4.1.3. *Community Mechanisms.* *Trichodesmium* spp. aggregates can contain from a few to several hundred individual trichomes. As trichomes within the aggregate grow and divide, or senesce and die, the balance between trichomes producing O_2 and trichomes removing O_2 can change. In addition to trichomes of *Trichodesmium* spp. there is a metabolically-active microheterotrophic community present within, and on, photosynthetically-active aggregates (Paerl *et al.*, 1989), the abundance of which may change throughout the life history of the aggregate. Cilliated and flagellated protozoan grazers are also closely associated with these aggregates (Borstad and Borstad, 1977), and represent an additional O_2 sink. The quantitative extent to which these O_2 removing activities of these various aggregate community members are able to depress aggregate internal $[O_2]$ is unknown. Further work should be directed towards observations of the relationship between numbers and quantities (or some relevant biomass parameter) of non-*Trichodesmium* members of an aggregate and the O_2 dynamics associated with the same aggregate.

4.2. CONCLUDING REMARKS

There appear to be a large number of mechanisms available to aggregates of *Trichodesmium* spp. for the regulation of internal O_2 concentrations. Aggregate O_2 concentrations are the result of a dynamic interplay between a number of O_2 consuming mechanisms at the physiological, physical (hydrodynamic, light shading and scattering) and community levels and photosynthetic O_2 production. The highly variable rates obtained for colony-specific carbon fixation (Table 1) and net O_2 evolution on a chl a specific basis (Fig. 5) are strong evidence that the number of trichomes that are photosynthetically active within a given aggregate may be highly variable. If we assume heterogeneity or individuality among the rates of any or all of the above mechanisms we have created the rationale for the observed high degree of individuality of O_2 dynamics that exist in aggregates of *Trichodesmium*. Furthermore, we can develop a mechanistic basis for suspecting that photosynthetic production and diazotrophy dynamics may vary substantially, if not dramatically, among aggregates, either on population or community levels.

It remains to be demonstrated definitively, but it is tempting to speculate, that a high degree of O_2 consumption within an aggregate could lead to greater rates of diazotrophy for that particular aggregate. The net effect of contemporaneous high rates of oxic photosynthesis and the ability to fix dinitrogen gas into a biologically available form (either at the same time in individual trichomes or individual aggregates) is a unique ability for this organism to contribute significantly to planktonic production and N flux in the open ocean. Fogg's original microzone hypothesis has led to a substantial number of useful and uniquely appropriate studies aimed at examining and elucidating small-scale (microzone) O_2 dynamics and their impacts on microbial production and nutrient cycling processes in these aggregates. The individuality observed in oxygen dynamics amongst aggregates (should it correlate with diazotrphic ability) would represent a novel, unique, and in retrospect effective (ingenious) strategy for optimizing growth in radiant energy-rich, N-deplete oligotrophic ocean waters.

5. Acknowledgements

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GRAZERS AND ASSOCIATED ORGANISMS OF *TRICHODESMIUM*

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ABSTRACT. Colonies of the pelagic cyanobacterium *Trichodesmium* consist not only of filamentous trichomes, but of other types of associated organisms as well, including bacteria, diatoms, dinoflagellates, fungi , protozoa, hydrozoans and copepods. These associated organisms use *Trichodesmium* as a physical substrate and as a food source. Pelagic harpacticoid copepods (the genera *Macrosetella* and *Miracia* in particular), are the only organisms that have been quantitatively shown to use *Trichodesmium* as a food source. There have been anecdotal reports of other organisms feeding on *Trichodesmium* including salps, crabs and fish. These micro-communities, with tight nutrient coupling, may be an adaptation for existence in oligotrophic tropical and subtropical seas. Associated organisms may cause physiological changes in *Trichodesmium*, which could account for much of the inter-colony variation when measuring various physiological parameters of this cyanobacteria. *Trichodesmium* can make a significant contribution to "new production" of nitrogen and the flux of organic matter from surface waters. Therefore, the consumption and fate of *Trichodesmium* has important consequences for understanding the inputs and outputs of carbon and nitrogen in the open ocean.

1. Introduction

In the open ocean, physical substrates are often a scarce commodity. It is therefore not surprising that colonies of the ubiquitous tropical and subtropical cyanobacteria, *Trichodesmium*, become "rafts" for many different types of organisms. The colonies act not only as physical substrates but also as oases of organic compounds. These open ocean microcosms have been likened to miniature *Sargassum* communities (Borstad 1978a; Roman 1978). The complex interactions of *Trichodesmium* and its associated organisms have not been fully investigated. Basic questions such as what eats *Trichodesmium*; how recycling of nutrients occurs within the micro-community; and what determines the trophodynamic fate of nitrogen and carbon from *Trichodesmium* colonies, remain unanswered. Most physiological studies of *Trichodesmium*

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ignore the fact that colonies are not just composed of cyanobacterial filaments, but are a complex community of autotrophs and heterotrophs. Organisms associated with *Trichodesmium* may influence the physiological state of the cyanobacteria as well as the morphology of the colony. Much of the considerable variability associated with measurements of *Trichodesmium* physiological features could result from differences in colonization or epiphytization of individual colonies (Geiselman 1977; Borstad 1978a). The attachment or grazing by animals undoubtedly affects photosynthetic and nitrogen fixation rates as well as nutrient concentrations surrounding the colonies and has direct relevance to the ecology of *Trichodesmium* (Geiselman 1977). Since whole colony physiology is affected by the associated organisms, metabolic studies should take the entire *Trichodesmium* community into consideration (Borstad 1978a).

2. Associated Organisms

2.1 BACTERIA

Bacteria are the most numerous organisms associated with *Trichodesmium* (Borstad and Borstad 1977). Dense assemblages of bacteria are sometimes concentrated at the cell junctures (Carpenter and Price 1976). "Significant" bacterial populations were found on 85% of both spherical (puffs) and parallel (tufts) *Trichodesmium thiebautii* colonies surveyed in a Barbados study (Borstad 1978b). Direct microscopic counts gave estimates of 50,000 bacteria per colony including members of *Pseudomonas*; *Corynebacterium*; *Bacillus*; *Micrococcus*; *Acinetobacter*; *Cytophaga* and *Flavobacterium*. Bacterial populations were "exceptionally dense" on 45% of the spherical *Trichodesmium* colonies and 16% of the parallel colonies examined by Borstad (1978b). This was probably the result of a more favorable habitat on the spherical colonies' complex mucilaginous polysaccharide matrix, which is conducive to the rapid development of large and varied bacterial populations (Borstad 1978b). The presence of increased numbers of bacteria may in fact add to this organic matrix.

A wide variety of heterotrophic bacteria were also found either attached to, or loosely associated with the mucilaginous matrix surrounding trichomes in Caribbean *Trichodesmium* (Paerl et al. 1989). Bacteria attached to the muccopolysaccharide layer may help keep the trichomes in colonial form. Bacteria break down complex organic compounds during heterotrophic growth, that may then be available for use by the cyanobacteria or other community associates (Lange 1971). Inorganic nitrogen, vitamins, and chelating agents may also be made available by bacterial metabolic activity. Release of these compounds could induce morphological changes in cyanobacteria (Lange 1974). Bacterial respiration could also promote reduced microzones facilitating microaerophilic reactions (Paerl 1982, Paerl et al. 1989).

Trichodesmium collected from a healthy, physiologically active population of colonies at 25 m were found to have fewer bacteria than colonies collected from less active populations of colonies from the surface or 50 m (Borstad 1978b). An explanation for these results is that the population from 50 m was older and senescent, therefore probably excreting more extracellular material for bacterial growth as opposed to the more active younger colonies (Borstad 1978b). Hence, the age of the *Trichodesmium* colony and the extent of the remineralization taking place

it, may have an influence on the entire community structure. Paerl et al. (1989) noted that both epiphytic bacteria and the *Trichodesmium* host appeared physiologically sound, stating that: "Far from signaling antagonism, senescence and death, these associations hint of mutualistic, if not symbiotic, interactions within the *Trichodesmium* aggregates". There is strong selective pressure in oligotrophic systems where *Trichodesmium* exist, to maintain a tight nutrient coupling system. This bacterial-*Trichodesmium* association may in fact be somehow obligate (Paerl et al. 1989), as no one, as yet, has been able to obtain an axenic culture of *Trichodesmium* (Ohki and Fujita 1982).

2.2 CYANOBACTERIA

Recently there have also been reports of filamentous, 1 μm diameter cyanobacterial associates on and within *Trichodesmium* colonies, of the genus *Phormidium* (Siddiqui et al. this volume). These cyanobacteria may be similar to the filamentous 1 μm bacteria that Paerl et al. (1989) described. Unicellular cyanobacteria have also been seen in association with *Trichodesmium* (Borstad and Brinckman-Voss 1979). The possible influences that other associated cyanobacteria may have on the *Trichodesmium* colony at the physiological or ecological level have not yet been elucidated.

2.3 DIATOMS

Eleven genera and 17 species of pennate diatoms have been found in association with *Trichodesmium*, including *Nitzschia*; *Synedra*; *Navicula*; and *Amphiprora*; often in abundances up to 300 cells per colony (Borstad and Borstad 1977). The diatoms attached to *Trichodesmium* were very lightly silicified, an adaptation to a pelagic existence, similar to diatoms found attached to *Sargassum* (Borstad 1978a).

2.4 DINOFLAGELLATES

Almost all of the spherical colonies of *Trichodesmium thiebautii* with mucilaginous matrices examined in the N. Atlantic near Barbados, contained nonmotile stages of dinoflagellates, including *Peridinum trochoideum*; *P. avellana* and *Gymnodinium* (Borstad 1978a). *Trichodesmium* may harbor cysts of the dinoflagellates during its temporary non-motile phase (Borstad 1978a). McCarthy and Carpenter (1979) found that 8% of the total chlorophyll in *Trichodesmium* colonies was chlorophyll *c*, indicating the presence of non-cyanobacterial pigment.

2.5 FUNGI

Two saprophytic species of fungi, a phycomycete and a chytrid have been described on *Trichodesmium* (Borstad and Borstad 1977). It has been suggested that the fungi may compete with bacteria for nutrients from the polysaccharide matrix or dead cells (Borstad 1978a).

2.6 PROTOZOA

Several types of microphagous or saprophytic protozoa have been described from *Trichodesmium* (Borstad and Borstad 1977). Ciliates can be abundant, including species from the families Tetrahymenida and Hypotrichida. Flagellates were reported as numerous as the ciliates, but less diverse (Borstad 1978a). The protozoa seem to inhabit the surface of the colonies and graze the periphery of the muccopolysaccharide layer (Borstad 1978a). Twenty four percent of the spherical colonies of *T. thiebautii* had protozoa compared to 14% of the parallel colonies in Borstad's (1978a) Barbados study. It has been suggested that the abundance of protozoa may vary in relation to number of bacteria (Borstad 1978a), and in fact Amoeba associated with *Trichodesmium* have been observed grazing bacteria that were growing among the filaments of the cyanobacterial colonies (Anderson 1977). The amoeba also contained *Trichodesmium* filaments within its digestive vacuoles, however this may occur incidentally while engulfing the bacterial prey (Anderson 1977). Phytoflagellates, colorless polyflagellates and nassellarian Radiolarians have also been observed among the *Trichodesmium* filaments (Anderson 1977). Borstad and Borstad (1977) also note that when normally free-living ciliates or flagellates are found in association with *Trichodesmium*, they are very abundant, suggesting rapid growth in response to the favorable environment encountered.

2.7 HYDROIDS

A previously undescribed colonial hydroid was observed on *Trichodesmium* from the Sargasso Sea (Geiselman 1977) and has since been assigned a new genus and species: *Pelagiana trichodesmiae* (Borstad and Brinckmann-Voss 1979) (Fig.1). Geiselman (1977) found that 40% of the near surface samples of *Trichodesmium* examined contained these hydroid inclusions. Hydroid polyps accounted for 93-98% of the ATP content of the colony, however, the filaments of these colonies appeared to be moribund (Geiselman 1977). The hydroid appeared to be associated with the mucilaginous matrix and was only found in association with the spherical colonies of *Trichodesmium thiebautii* (Borstad and Borstad 1977; Borstad and Brinckman-Voss 1979). It may be absent in the parallel form of *Trichodesmium thiebautii* due to the weaker colonial structure and lack of mucilaginous matrix (Borstad 1978a). *Pelagiana trichodesmiae* is a voracious feeder and has been observed with both adult and naupliar copepod stages as well as chaetognaths in their guts (Borstad 1978a; Borstad and Brinkman-Voss 1979). Such predation may influence community structure, as well as *Trichodesmium* physiology.

2.8 COPEPODS

The most phylogenically advanced organisms associated with the *Trichodesmium* microcommunity are pelagic harpacticoid copepods (Bjornberg 1965; Roman 1978; Bottger-Schnack and Schnack 1989). The majority of harpacticoids are benthic and have creeping juvenile, naupliar stages, that require physical substrate (Fig. 2). While culturing three species of Miracidae copepods: *Macrosetella gracilis*; *Oculosetella gracilis*; and *Miracia efferata*, Bjornberg (1965) noted that the addition of small tufts of *Trichodesmium thiebautii* greatly

improved the health of the nauplii. Adult *Macrosetella gracilis* have been found to ingest *Trichodesmium*; whether the nauplii derive nutrition from *Trichodesmium* directly has not yet been determined (Bjornberg 1965; Roman 1978; discussed in sect. 3.3).

Macrosetella gracilis nauplii were not only attached to *Trichodesmium* filaments in the lab, but were also found attached to *Trichodesmium* from the coastal and shelf waters off the coast of southern Brazil (Bjornberg 1965). Calef and Grice (1966) similarly found a strong correlation between the abundance of *Trichodesmium thiebautii* and *Macrosetella gracilis* off the coast of northeastern South America. In addition, Tokioka and Bieri (1966) found *Macrosetella gracilis* nauplii in association with *Trichodesmium erythraeum* off the coast of Seto, Japan, in densities of 1 - 6 per colony. Cyclopoid and harpacticoid copepods have also been observed in association with a bloom of *Trichodesmium erythraeum* in Tonga (Bowman and Lancaster 1965).

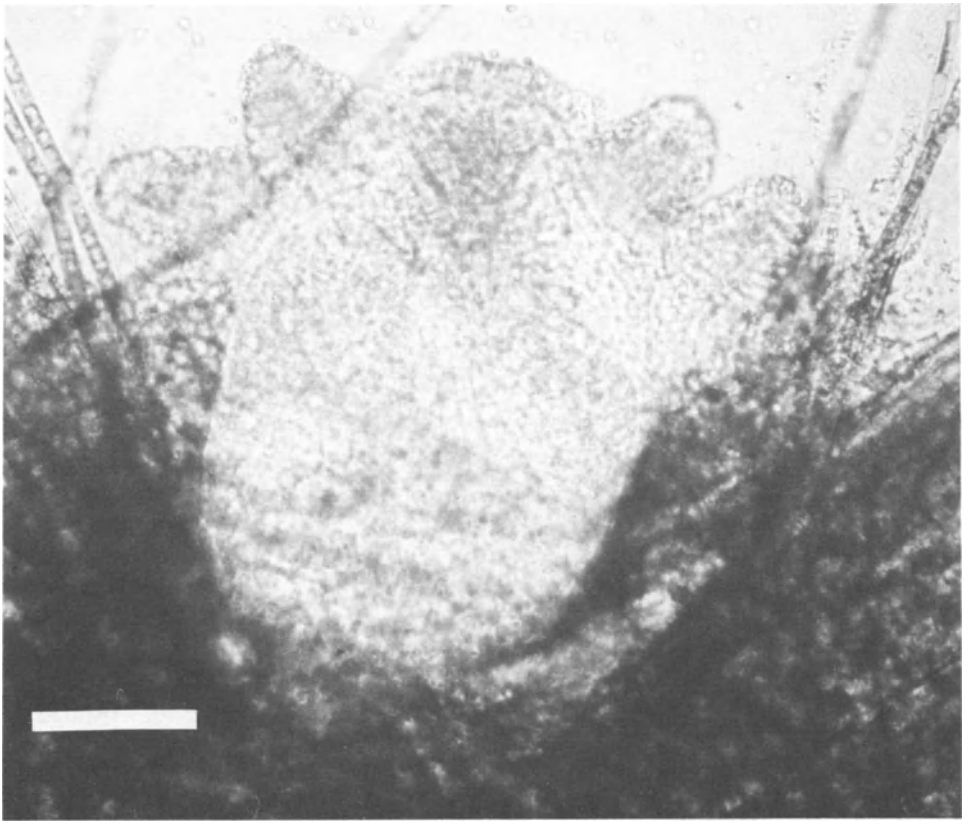

Figure 1. Hydroid *Pelagiana trichodesmiae* on a spherical colony of *Trichodesmium thiebautii* from the Eastern Caribbean (Scale bar = 50 µ).

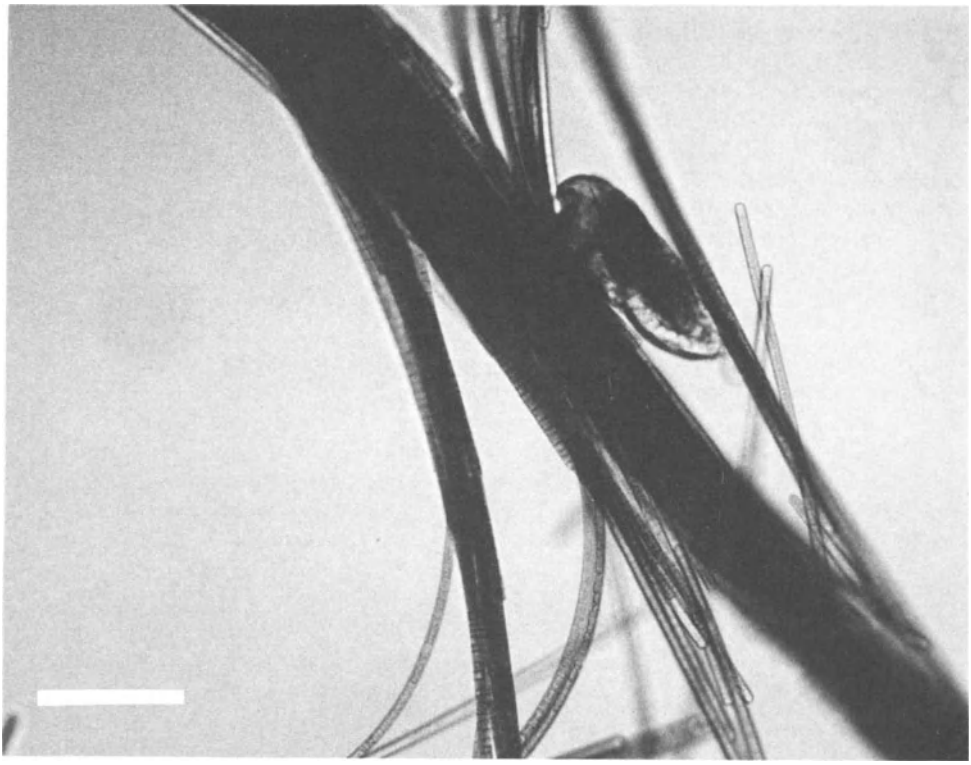

Figure 2. Harpacticoid copepod nauplii of the genus, *Macrosetella gracilis* attached to a parallel colony of *Trichodesmium thiebautii* from the Eastern Caribbean (Scale bar = 10 μ).

Bjornberg (1965) described a specialized hook-like appendage on the second and third appendages of *Macrosetella gracilis* nauplii, which it apparently uses to attach to cyanobacterial filaments and to move along the filaments in a "waddling" motion. Four percent of all the colonies inspected in the N. Atlantic, off Barbados, contained nauplii (Borstad 1978a). They were more common on spherical colonies (7%) than on parallel colonies (2%). The difference may result from greater mobility afforded the nauplii on parallel colonies, and that the spherical colonies often harbor the hydroid *Pelagiana*; a potential predator to the copepod (Borstad 1978a).

Since harpacticoid copepods are generally benthic and have poor swimming ability compared to calanoid and cyclopoid copepods, the association of *Macrosetella* with *Trichodesmium* filaments can be viewed as a necessary adaptation to life in the open ocean (Bjornberg 1965). Although adult *Macrosetella* can exist independently of *Trichodesmium* filaments, its swimming is "slow and ungraceful" (Bjornberg 1965). However, when adults or copepodites are attached to a *Trichodesmium* they glide forward more easily, using the filament as a "sledge" (Bjornberg 1965, O'Neil, personal observation).

There are several other species of harpacticoid copepods which likely utilize *Trichodesmium*. *Miracia efferata* also clasps onto particulate matter in the plankton, however the specific substrate preferences for the nauplii have not been determined (Bjornberg 1965). *Miracia efferata* adults are often found in association with *Trichodesmium* and will ingest the cyanobacterium (O'Neil and Roman unpubl. data; discussed in sect. 3.3). Other pelagic harpacticoid copepods found in tropical and subtropical waters that are likely to utilize *Trichodesmium* are *Microsetella norvegica*, *Microsetella rosea*, *Miracia minor* and *Oculosetella gracilis*.

3. Grazing on *Trichodesmium*

The association of most organisms with *Trichodesmium* appears to be largely mutualistic. The associated organisms are provided with physical substrate and organic nutrients. The *Trichodesmium* may receive trace elements and inorganic nutrients. However, in some instances the epibionts may be be using the cyanobacterial filaments as a food source, and larger planktonic and nektonic organisms may also graze on *Trichodesmium* colonies. Cyanobacteria in general, are relatively poor food sources compared to other phytoplankton, as they often contain toxins and are nutritionally inadequate (Porter and Orcutt 1980), especially in terms of some essential fatty acids (Wood 1974, Harwood et al. 1988). Also, filamentous forms are often difficult for grazers to ingest (Porter and Orcutt 1980). These attributes often result in a greater expenditure of energy by the grazer in manipulation, rejection, digestion and detoxification, than is gained by the ingestion of the cyanobacterium (Porter and Orcutt 1980). McCarthy and Carpenter (1979) concluded that *Trichodesmium* must be subjected to little grazing pressure to account for their slow growth rate and high standing stock in the Sargasso Sea. One reason may be a highly neurotoxic compound found in some populations of *Trichodesmium* (Hawser et al. 1991).

3.1 CRABS AND FISH

Most accounts of organisms grazing on *Trichodesmium* are rather qualitative and anecdotal. As reported in a review article by Carpenter (1983), the first account of *Trichodesmium* being consumed, was that of Hentschel (1922) who noted masses of *Trichodesmium* in the guts of the *Sargassum* crab *Planes minutus* in the North Atlantic Ocean. Carpenter (1983) reviewed several reports of ingestion of *Trichodesmium* by fishes including Indian Ocean Clupeid species: *Sardinella gibbosa* (Devanesan 1942) and *Hilsa kanagurta* (Ramamurthy 1970a); a species of the mullet family Muglidae: *Mugil waigiensis* (Devanesan 1942); and a mackrel species of the family Scombridae: *Rastrelliger kanagurta* (Ramamurthy 1970a). A study on the seagull, *Laurus brunicephalus*, that feeds on two species of these fish, showed that 70-75% of the gull's gut contents consisted of *Trichodesmium erythraeum*. The gut contents also had reduced bacterial content, suggesting antibacterial activity resulting from *Trichodesmium erythraeum* (Ramamurthy 1970b). Previous studies have shown that *Trichodesmium erythraeum* exhibits anti-bacterial properties against both gram positive and gram negative bacteria (Ramamurthy

and Krishnamurthy 1967). It is possible that ingestion of *Trichodesmium* is incidental to fish feeding in bloom-laden water. A tuna species, *Katsuwonus pelamis*, prevalent in pre-bloom waters, reportedly avoided a *Trichodesmium erythraeum* bloom in the Arabian Sea, returning after disappearance of the cyanobacteria (Nagabhushanam 1967). The author stated that it was not known whether the avoidance mechanism was mechanical or biochemical (Nagabhusham 1967), however it may have been due to the neurotoxin found in *Trichodesmium* (Hawser et al. 1991).

3.2 SALPS

Nemoto (1975) reported on the possibility of two species of salps, *Salpa fusiformis* and *Weelia cylindrica*, grazing on *Trichodesmium* in the Pacific Ocean off Japan. Salps have been observed in the Caribbean with *Trichodesmium* filaments in their guts, and often there seems to be an inverse relationship in abundance of salps and *Trichodesmium* colonies (O'Neil, personal observation). More rigorous experiments to document whether salps do in fact digest, and not just ingest *Trichodesmium*, need to be conducted.

3.3 COPEPODS

The association of harpacticoid copepods, *Macrosetella gracilis* in particular, is a common attribute of *Trichodesmium* colonies (Bjornberg 1965; Calef and Grice 1966; Tokioka and Bieri 1966; as discussed in sect. 2.8). The creeping nauplii seem to require *Trichodesmium* as a substrate, as they have not been found elsewhere (Bjornberg 1965). Different opinions exist on whether the juvenile stages actually feed on the *Trichodesmium*, or if they even feed at all during these stages (Krishnaswami 1951; Borstad and Borstad 1977; Bottger-Schnack 1989). Evidence that nauplii may actually feed, was shown in a study attempting to culture *Trichodesmium* (Ohki et al 1991), that found all the cyanobacterial colonies disappeared within a week. Subsequent microscopic analyses revealed that 99% of the *Trichodesmium* colonies from the Kuroshio current, where original samples were collected, had *Macrosetella gracilis* nauplii attached. Removal of these copepods resulted in longer survival times of the *Trichodesmium* (Ohki et al. 1991).

The feeding of adult *Macrosetella* is described by Bjornberg (1965): "they eat a filament of *Trichodesmium* slowly, (as if sucking it in) whilst clasping other filaments alongside of the one being eaten". Actual ingestion rates of *Trichodesmium* were determined for adult *Macrosetella gracilis* collected from the Gulf Stream off Miami by measuring chlorophyll disappearance in experimental flasks (Roman 1978). The overall mean ingestion rate was 1.08 μg C μg copepod C^{-1} d^{-1}. This corresponds to *Macrosetella gracilis* ingesting 90-126 % of its body carbon per day when feeding on *Trichodesmium* (Roman 1978). These results have been confirmed using ^{14}C radiolabelled *Trichodesmium* from the Sargasso Sea (Roman unpubl. data) and the Caribbean (O'Neil and Roman unpubl. data). Recent studies indicate that *Miracia efferata*, another open ocean harpacticoid, also feeds on *Trichodesmium* (O'Neil and Roman unpublished data). It is also intersesting to note that the harpacticoid copepods that have been shown to graze on *Trichodesmium*, *Macrosetella gracilis* and *Miracia efferata*, show tolerance to the

cyanobacteria's concentrated toxin at concentrations that are lethal to other zooplankton (Hawser, Codd, O'Neil and Roman, in prep).

4. Food Web Dynamics

Possible interactions between organisms which colonize *Trichodesmium* were addressed by Borstad (1978a). These interactions (Fig. 3) include both inorganic nutrient uptake and release, dissolved organic release and uptake and grazing. Although exact rates of interchange are not known, we can speculate that the end result is to efficiently recycle nutrients, giving *Trichodesmium* and its associated organisms an advantage in oligotrophic waters.

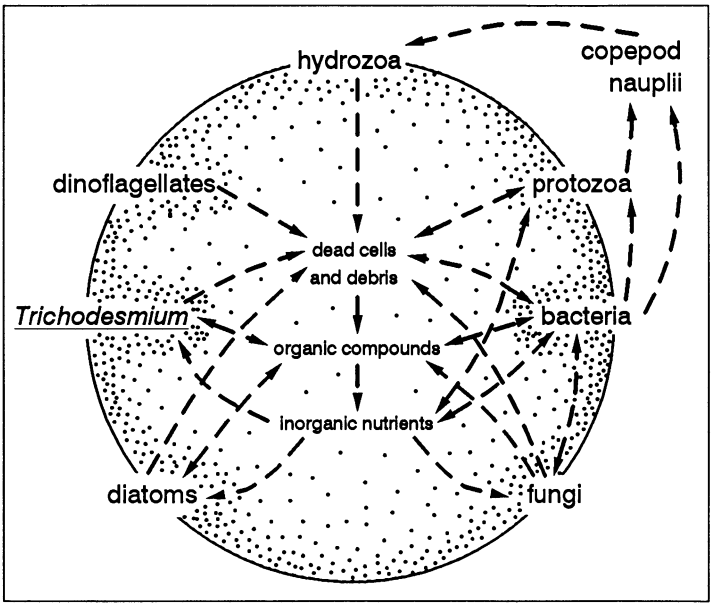

Figure 3. Summary of possible interactions between organisms within the *Trichodesmium* community. Shaded area represents physical confines of the matrix in which organisms are embedded. (Reprinted with permission from G.A. Borstad (1978) PhD thesis; Fig. 5.3).

Active remineralization is also carried out by fungi and protozoa (Borstad 1978a). Much of the organic matter accumulated in the *Trichodesmium* colony as bacterial biomass becomes food for protozoa (Borstad 1978a). Borstad and Borstad (1977) reported copepods and chaetognaths in the guts of the hydroid *Pelagiana trichodesmia*, an observation which may indicate a tight trophodynamic coupling within the microcommunity. Harpacticoid copepods may act as an important agent for secondary production from *Trichodesmium* (Fogg 1982).

The release of photosynthetic products has been shown to be dependent on the physiological state of the colony (Shimura et al. 1978). The question of physiological state also comes into consideration in determining what factors trigger bloom formation and decay in *Trichodesmium* (Paerl et al. 1989). The physiological state of the cyanobacteria could also play an important role in determining the assemblage of associated organisms, as well as the trophodynamic interaction of the *Trichodesmium* microcommunity. Paerl et al. (1989) pose the pertinent question: when studying "naturally-occurring consortial interactions... what controls, or biotic equilibria exist that help maintain the fine-line between observed mutualistic interactions and potential opportunism or antagonism?". They suggest that when this 'fine line', or delicate balance is disturbed, the *Trichodesmium* community may change from a balanced mutualistic association, facilitating the exchange of essential metabolites and nutrients to a stress induced antagonistic, or disruptive association (Paerl et al. 1989).

Autotrophic carbon fixation by *Trichodesmium* can be regarded as "new" production because *Trichodesmium* fixes N_2 (Dugdale and Goering 1967). Carbon fixation by *Trichodesmium* can contribute 20% of phytoplankton production in the Caribbean (Carpenter 1983). In oligotrophic waters, "new" production by phytoplankton is generally 10% of total phytoplankton production (Eppley and Peterson 1979). Thus, where it is abundant, the contribution of *Trichodesmium* to new production and the flux of organic matter from surface waters, can equal that of the other phytoplankton. Therefore, the consumption and fate of *Trichodesmium* has important consequences for understanding the inputs and outputs of carbon and nitrogen in the ocean. Macrograzers such as harpacticoid copepods facilitate the flux of organic material to deep water through the production of rapidly sinking fecal pellets. In addition, by ingesting *Trichodesmium*, the fixed carbon and nitrogen is sequestered into animal biomass where it can be passed on to higher trophic levels. This is especially important in oligotrophic waters where carbon fixation and nitrogen inputs are low. Additional research is needed to address food-web dynamics which control the production, consumption and fate of *Trichodesmium*.

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TROPHODYNAMICS OF MARINE CYANOBACTERIA BLOOMS

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ABSTRACT. Although cyanobacteria blooms can dominate annual production and phytoplankton biomass in some marine regions, little is known on the fate of these large aggregations of autotrophic, surface-dwelling prokaryote populations. The importance of bloom-forming *Trichodesmium* spp. and *Nodularia spumigena-Aphanizomenon flos-aquae* in carbon and nutrient dynamics of tropical and sub-arctic regions, respectively, will be discussed. The limited collected data indicate that surface blooms funnel C, N and P through an active microbial loop, leading to substantial regeneration in surface waters.

1. Introduction

Trichodesmium and *Nodularia-Aphanizomenon* dominate surface waters of the tropics and Baltic Sea during blooms that can cover several meters to 90,000 km^2 (e.g., Dupouy et al. 1988). These surface aggregations of autotrophic prokaryotes occur during warm, stable conditions when phosphorus is present and nitrogen is undetectable. The surface blooms of these algae are characterized by large aggregations of colonies of intertwined or clumped trichomes, supporting high nitrogen fixation rates as a significant source of fixed nitrogen in their respective areas. The colonies, in turn, provide an ideal microenvironment for a menagerie of protozoans, diatoms, dinoflagellates, flagellates, fungi, ciliates, rotifers, juvenile and adult copepods and cladocerans, polychaete larvae and fish eggs and ichthyoplankton.

As huge reservoirs of fixed carbon, nitrogen and phosphorus, blooms of *Trichodesmium* and *Nodularia-Aphanizomenon* should prove to be the principal substrate for production in higher trophic levels of both systems as well as the primary source of fixed nitrogen for the nitrogen-impoverished systems these taxa frequent. *Trichodesmium* is responsible for 4.8 Tg annually, one-quarter of total nitrogen fixation in the sea (Capone and Carpenter 1982). In 1974, Baltic prokaryotes were, in turn, responsible for 10^5 tons of new nitrogen, equal to total input of nitrogen from land in the region (Rinne et al. 1981). However, carbon transfer and flux associated with these abundant prokaryotes has not been as rigorously examined.

The purpose of this review is to summarize existing information on the fate of *Trichodesmium* and *Nodularia-Aphanizomenon* blooms, addressing the flux of carbon and nutrients in waters hosting the bloom species. As will become evident in the review, little quantitative data exists on the importance of these production maxima in energetics and nutrient dynamics of the respective systems.

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2. *Trichodesmium*

This marine cyanobacterium forms blooms in the Caribbean, North and South Atlantic Oceans, the North Pacific and Indian Oceans and the Red Sea (see Carpenter, 1983). The alga concentrates at the surface in spherical or fusiform colonies, often containing 40-60 to several hundred trichomes. Spherical or radial colonies contain large quantities of mucilage at their center while the fusiform colonies are free of a mucilage matrix (Borstad and Borstad 1976). Each trichome is surrounded by a mucilaginous sheath (see Paerl et al. 1989) with varying degrees of bacterial attachment.

The chemical composition of *Trichodesmium* has been summarized by Carpenter (1983). Carbon and nitrogen content of Atlantic and Pacific colonies were similar with 9.7-11.6 μgC and 2.09-2.4 μgN·colony^{-1} (Mague et al. 1977; McCarthy and Carpenter 1979). Overall composition differed from expected Redfield ratio, with C:N:P ranging from 80:20:1-891:125:1 in the North Pacific (Mague et al. 1977; Karl et al. this volume) to 188:21:1 for *Trichodesmium*-dominated plankton from the Red Sea (Naqvi et al. 1986). Low phosphorus content was also reflected in high C:ATP ratios (540-650, 800) in North Atlantic and Pacific Ocean blooms, respectively, as well as in measurable alkaline phosphatase activity in North Atlantic Ocean colonies (McCarthy and Carpenter 1979; Karl et al. this volume).

2.1. *TRICHODESMIUM*-BACTERIA INTERACTIONS

Trichodesmium colonies are characterized by large bacterial populations with motile rods actively moving around the colony and trichomes and an attached assemblage of rods and filamentous (200 μm) forms (see Paerl et al. 1989). High bacterial densities have been routinely observed in *Trichodesmium* (Steven and Glombitza 1972; Borstad and Borstad 1976; Mague et al. 1977; Borstad and Brinckmann-Voss 1979; Borstad 1982; Paerl et al. 1989; Paerl 1991) and are the base of a complex microbial food web in the colonies.

In an extensive review of cyanobacterial-bacterial interactions, Paerl (1990, 1991) and Paerl and his collaborators (1989) have argued strongly for a mutualistic association between bacteria and *Trichodesmium* in the natural environment. A strong case has been presented for viability of each phylum as a dependent of the other: *Trichodesmium* supplies habitat and a source of organic matter as mucilage, photosynthetic exudates and fixed nitrogen for the heterotrophic bacteria while bacterial respiration and heterotrophy, in turn, provide inorganic carbon for continued photosynthesis and reduced oxygen tensions for nitrogen fixation and recycled nutrients for subsequent reincorporation by the alga. This mutualistic relationship typifies maximum growth rates of the alga and is not symptomatic of bacterial-mediated decomposition of the alga, possibly evident for *Nodularia-Aphanizomenon* blooms in the Baltic (see below). The transfer of fixed ^{15}N from *Trichodesmium erythraeum* to associated microbes (Bryceson and Fay 1980) indicates the direct benefit for the bacteria in the association.

Organic substrates for the associated bacterial community include mucilage of the colonies and trichomes and excreted low molecular weight photosynthetic products. The mucilage observed in spherical colonies is a carboxylated polysaccharide produced either by *Trichodesmium* or its associated guests (Borstad and Borstad 1976; Borstad 1978a, 1982; Borstad and Brinckmann-Voss 1979). Similarly, individual trichomes are covered by a mucilaginous sheath intertwined with attached rod and filamentous bacteria (Paerl et al. 1989). Although bacterial ingestion of this material has not been estimated, bacteria can produce exoenzymes for the hydrolysis of polysaccharides (e.g., Meyer-Reil 1984) followed by the subsequent uptake of the lower molecular weight carbohydrates.

Junctions between cells are apparently sites of organic excretion as bacteria were concentrated at these sites in *Trichodesmium* collected in the Caribbean Sea (Carpenter and Price 1977); less than 1% of the surface population, while 8.3% of the *Trichodesmium* population at 50 m, was infected in this manner. Borstad (1978a) also identified these junctions as active sites for the release of carboxylated polysaccharide.

Carbon fixation and subsequent release of dissolved organic carbon (DOC) in healthy *Trichodesmium* can also supply substrate. *Trichodesmium* from the North Pacific Ocean released approximately 5-8% of recent photosynthate (Shimura et al. 1978), comparable to release observed in healthy eucaryotic phytoplankton assemblages (e.g., Sharp 1977; Sellner 1981). *Trichodesmium* isolated from off Barbados released from 0-18% of recently fixed carbon (Borstad 1978 a,b). Low molecular weight materials in the released pools could be readily assimilated by the heterotrophic bacteria associated with the colonies as exemplified in microautoradiographs of *Trichodesmium* communities exposed to labelled fructose, glucose, mannitol and amino acids (Paerl et al. 1989). Considering that carbon fixation rates are low in *Trichodesmium*, 2.25 $pg \cdot [cell \cdot h]^{-1}$ (Carpenter and Price 1977), maximum release of photosynthate would approximate 0.4 $pgC \cdot [cell \cdot h]^{-1}$ or on the order of 2.2 $\mu gC \cdot [colony \cdot 14\ h\ d]^{-1}$ for a colony of $38.7 \cdot 10^3$ cells (McCarthy and Carpenter 1979). Additional organic substrate would arise from microbial loop activities within the colonies (sloppy feeding, excretion, lysis, etc.), providing additional carbon for maintaining the active bacterial assemblages of the trichomes.

Many investigators report that natural *Trichodesmium* blooms are often decaying or in poor condition during sampling (Brongersma-Sanders 1957; Qasim 1970; Steven and Glombitza 1972; Geiselman 1977; Borstad 1978a, 1982; Devassy et al. 1978; Li et al. 1980; Nair et al. 1980; Creagh 1985; Devassy 1987). The release of organic matter from unhealthy or lysing cells (possibly mediated by fungi, Borstad and Brinckmann-Voss 1979 or planktonic viruses; see Overview) should be substantially greater than from healthy populations. However, the quantities of DOC and DON appearing during decay and lysis of *Trichodesmium* are not available. Considering that average cellular C and N contents approximate 150 and 27 pg (from Marumo 1975 in Carpenter 1983) and that colonies might contain $3 \cdot 10^4$ cells (McCarthy and Carpenter 1979), lysis of non-productive colonies could provide 4.5 μgC and 0.81 μgN to the associated microbial flora. This C and N contribution is in addition to mucilage and exudates produced during normal growth of the alga.

2.2. *TRICHODESMIUM*-ZOOPLANKTON INTERACTIONS

The longevity and spatial extent of *Trichodesmium* blooms coupled with their slow growth rates indicate only modest zooplankton grazing should typify blooms at least in the Sargasso Sea (McCarthy and Carpenter 1979). However, investigators have hypothesized similar minimal losses to zooplankton in other systems as well (Borstad and Borstad, 1976; Nair et al. 1980; Shaikh et al., 1986; Devassy 1987; Paerl 1991).

One copepod requires *Trichodesmium* as nursery and food source. Early observations suggested strong linkages between densities of *Macrosetella gracilis* and *Trichodesmium* abundance. Calef and Grice (1966) noted a strong positive correlation between densities of *T. thiebautii* and the harpacticoid at 34 stations off the northeast coast of South America; a similar relationship has been noted in the Red Sea (Böttger-Schnack and Schnack 1989). Ohki et al. (1991) have noted that more than 99% of the *Trichodesmium* colonies collected from surface waters of the Kuroshio Current were infected with *Macrosetella*. Björnberg (1965) noted that nauplii of the copepod continued to develop in culture if trichomes of *T. thiebautii* were added, supporting observations of high *Macrosetella* nauplii densities in *Trichodesmium* colonies collected in the Caribbean (Borstad and Borstad 1976). Direct observation of the ingestion of trichomes by the copepod was

observed by Björnberg (1965) who noted that adult *Macrosetella* would eat a filament slowly, as if sucking it in, whilst clasping on to other filaments immediately alongside. Ohki et al (1991) reported that on returning copepod-infested *Trichodesmium* to the laboratory, trichomes rapidly disappeared in culture, presumably due to grazing by the attached copepods.

Roman (1978) documented ingestion of *Trichodesmium* by the harpacticoid by measuring changes in *Trichodesmium* chlorophyll concentrations in the presence of the copepod. The copepod would ingest 90-126% of its body carbon·d^{-1} during feeding on the cyanobacteria, consuming 5.5 μgC and 1.2 μgN daily. Roman also noted that newly hatched nauplii and copepodites remained attached to *Trichodesmium* trichomes, continually feeding on individual cells. *Macrosetella* in the Gulf Stream off Florida would consume 0.14-2.75 mgC·$[m^2 \cdot d]^{-1}$ and 0.03-0.06 mgN·$[m^2 \cdot d]^{-1}$ from grazing on colonial *Trichodesmium*. More intensive experiments are currently in progress (see O'Neil and Roman this volume; R. Dawson, unpubl. data) and preliminary results indicate significant grazing pressure by the copepod on Caribbean *Trichodesmium* colonies as well as appearance of *Trichodesmium* accessory pigments in the gut of the copepod.

Macrosetella also ingests Red Sea *Trichodesmium* with the copepod's reproduction and vertical distribution closely tied to the abundance of the alga in surface waters (Böttger-Schnack 1989, 1991; Böttger-Schnack and Schnack 1989). Egg production and over-wintering success in the copepod is dependent on *Trichodesmium* in the water column and actively feeding and deep living overwintering adults are rich in oil droplets, perhaps indicating transfer of lipids from the cyanobacterium to the copepod.

There are only a few other references indicating *Trichodesmium* as a food source for zooplankton. In two studies of North Pacific plankton, ^{15}N enrichment was determined in phytoplankton dominated by *Trichodesmium* and in zooplankton of the immediate area (Wada and Hattori 1976; Mitsubushi-kasei report 1979). *Trichodesmium* $\delta^{15}N$ was low (-1.7 to 0.5 ppt and -0.8 to -2.1 ppt) due to little fractionation of ^{15}N in the atmosphere and subsequent incorporation during N-fixation. Zooplankton from depths overlapping with *Trichodesmium* and in the immediate region surrounding the alga had a similarly low $\delta^{15}N$ signature (2 and 2.1 to 3.7 ppt); zooplankton and micronekton from deeper layers devoid of *Trichodesmium* had higher $\delta^{15}N$ (4.5 to 7.5 ppt) indicating food supply was more likely higher $\delta^{15}N$ non-cyanobacteria ($\delta^{15}N$ approximates 7 for non N-fixing phytoplankton; Wada and Hattori 1976). These data imply that zooplankton were likely feeding on the oceanic cyanobacteria; however, species composition of the herbivores was not provided so taxa ingesting the cyanobacterium cannot be identified. Harpacticoid copepods (*Macrosetella*?) and juvenile *M. gracilis* have been observed in association with *Trichodesmium* in the Pacific Ocean (Bowman and Lancaster 1965; Tokioka and Bieri 1966).

Non-crustacean grazers also ingest the cyanobacterium. Salps (*Salpa fusiformis* and *Weelia cylindrica*) have been collected with *Trichodesmium* in their guts (Nemoto 1975 in Carpenter 1983). O'Neil and Rueter (pers. commun.) have observed an inverse correlation between North Atlantic salps and *Trichodesmium*. Finally, pelagic tunicates filter *Trichodesmium* from surrounding waters (Alldredge 1976) but the abundance of *Trichodesmium* might control appendicularian abundances. As *Trichodesmium* increases, the percentages of damaged filtering houses and the number of unoccupied houses increases, largely due to clogging and piercing of each house with the "burrlike" *Trichodesmium* trichomes. Therefore, increasing cyanobacterial densities should reduce grazing pressure by the pelagic tunicates through damage to houses and accompanying declining filtering activity.

2.3. *TRICHODESMIUM* INTERACTIONS WITH OTHER POTENTIAL GRAZERS

Trichodesmium colonies are large aggregation sites for *Macrosetella* and non-copepod

heterotrophs that include protozoans, fungi, ciliates, hydroids and fish and polychaete larvae. Borstad and Borstad (1976) reported that fungi colonizing *Trichodesmium* colonies from the Caribbean Sea ingested mucilage produced in the colony. They also identified two dinoflagellates, *Peridinium trichoideum* and *Gonyaulax* sp., in the matrix of the colony; both taxa have mixotrophic relatives in the Pyrrhophyta and might conceivably act as phagotrophic herbivores or bacteriovores. Kimor and Golandsky (1977) noted coincidence of maximum *Trichodesmium* densities and peak densities of two tintinnid species, *Epiplocylis undella* and *Petalotricha ampulla*, in the Gulf of Elat; however, food preference was not indicated.

Considerable debate exists on the importance of *Trichodesmium* to nekton. Following a *Trichodesmium erythraeum* bloom off Ceylon, Chacko (1942) reported massive mortality of holothurians, fishes, crabs, sea urchins and molluscs. There are several reports (Chidambaram 1942; Devanesan 1942; Devassy et al. 1978) of *Trichodesmium* in the guts and gills of planktivorous fishes (sprat *Sardinella gibbosa* and *S. albella*, gizzard shad *Anodoutosoma chacunda*, milk-fish *Chanos chanos* and mullet *Mugil waigiensis*) with one report relating the success of the fishery off India to *Trichodesmium* abundance (Devanesan 1942). Chance ingestion is suggested by the presence of only a few filaments in guts of other invertebrates and fishes (*Squilla*, *Therapon jarbua*, *Caranx* spp., *Trichiurus* sp.; Devassy et al. 1978). The gut contents of sea gulls (*Laurus brunicephalus*) contained 70-75% *Trichodesmium* after feeding on sardines (*Hilsa kanagurta*) and mackerel (*Rastrelliger kanagurta*) with *Trichodesmium* filling 80-90% of their gut volume (Ramamurthy 1970). Bryceson (1980 in Carpenter 1983) observed active ingestion of *Trichodesmium* colonies by juvenile clupeids placed in tanks. However, John and Menon (1942) and Nagabhushanam (1967) suggested that *Trichodesmium* blooms off India inhibited planktivorous fishes and other plankton and depressed tuna catches, respectively. Sinking appendicularian houses containing *Trichodesmium* could also be ingested by plaice and sardines (Alldredge 1976), transferring cyanobacterial carbon to these planktivorous nekton. *Trichodesmium* has also been observed in the gut contents of the Sargassum crab *Planes minutus* (Hentschel 1922 in Carpenter 1983). With the absence of detailed data in most of these reports, the magnitude of nektonic grazing of *Trichodesmium* production remains unknown.

These observations list potential grazers on *Trichodesmium* blooms. In general, however, most observers believe that the bacteria associated with the colonies are the basis for carbon and nutrient flux in *Trichodesmium* blooms, actively preyed on by the numerous bacteriovores in the assemblage, rather than direct transfer of *Trichodesmium* production to herbivores of the systems (Borstad and Borstad 1976; Paerl et al. 1989; Paerl 1991).

2.4. SEDIMENTATION OF *TRICHODESMIUM*

The end of periodic eucaryotic phytoplankton blooms, as, for example, spring diatom blooms in temperate and sub-polar regions, is often typified by rapid sedimentation to bottom sediments (e.g., Riley 1956; Von Bodungen et al. 1975; Malone et al. 1988). Gas vacuoles in *Trichodesmium* prevent sedimentation from surface aggregations probably accounting in the persistence of surface blooms for 3 months in the Red and Arabian Seas (Nair et al. 1980; Shaikh et al. 1986). External pressures required to collapse gas vacuoles in *Trichodesmium* range from 12-37 bars (Walsby 1978), suggesting hydrostatically-induced sedimentation is unlikely. Sedimentation of *Trichodesmium* colonies has been postulated for colonies heavily infested with the attached hydroid *Pelagiana trichodesmiae* (Borstad and Brinckmann-Voss 1979); however, hydroid-induced sinking in *Trichodesmium* has not been documented. Similar arguments have been postulated for *Nodularia-Aphanizomenon* blooms in the Baltic (see below).

Dense aggregations of *Trichodesmium* at depth are rare. *Trichodesmium* has been collected at 175 m in the Caribbean (Borstad 1978a), 135 m in the North Atlantic (R. Dawson, pers.

commun.), 150-240 m in the North Pacific (Marumo and Asaoka 1974) and similar depths of the Red Sea. However, densities are in general low at depth. Kromkamp and Walsby (this volume) present convincing evidence for control of vertical distributions of *Trichodesmium* through synthesis and consumption of carbohydrate ballast in the alga. They conclude that colonies can sink to 100-200 m but most often, buoyancy regulation maintains the colonies in surface waters. Sediment trap collected particulates collected in the North Atlantic Ocean have not included *Trichodesmium* in the list of identified phytoplankton, although Honjo (1980) reports 'epiphytes' in material collected from the Sargasso Sea. The transport of *Trichodesmium* to subsurface depths could occur as inclusions in sinking discarded appendicularian houses in tropical oceans. As noted above, Alldredge (1976) has observed *Trichodesmium* in discarded houses. Through house sinking (~ 100 m·d^{-1}; Silver and Alldredge 1981; Taguchi 1982; Gorsky et al. 1984), a small fraction of surface bloom production could be delivered to sub-surface waters. Zeaxanthin-rich particulates have been identified in depths immediately above the deep chlorophyll maximum off the western coast of Africa and immediately beneath *Trichodesmium*-rich surface waters (Gieskes and Kraay 1986). However, the zeaxanthin has not been specifically attributed to sedimenting *Trichodesmium* trichomes or colonies; alternatively, the pigment could be in *Synechococcus* populations that typically inhabit these depths in oceanic systems (e.g., Prezelin et al. 1986).

2.5. *TRICHODESMIUM* AND INORGANIC NUTRIENT RECYCLING

Many investigators conceptualize the *Trichodesmium* colony and associated biota as a micro-habitat for nutrient recycling from the active microbial loop in the colony (Borstad 1978a,b; Bryceson and Fay 1980; see Paerl 1991). Blooms of the cyanobacterium typically coincide with undetectable inorganic nitrogen concentrations and slightly elevated phosphorus levels in the water column. Following development and subsequent decline of the bloom in surface waters, nutrients should be regenerated from the decay of the alga through vigorous bacterial and microheterotrophic metabolism. Detection of elevated nutrients following *Trichodesmium* blooms has been observed in coastal waters of India and north of the Hawaiian Islands and in samples collected from off Barbados; other blooms in the Atlantic, Pacific and Caribbean have less frequently been characterized by enhanced nutrient levels in post-bloom waters.

Off India, Devassy (1987) and Devassy and co-workers (1978, 1979) reported that inorganic phosphorus, nitrate and ammonium increased to 30, 1-2 and 0.5-2 μg-at·L^{-1} in the Arabian Sea following decay of *Trichodesmium*; diatoms replaced the cyanobacteria only to be followed by the cladoceran *Evadne* and dinoflagellates and chlorophytes. These taxa, in turn, were followed by *Noctiluca* giving rise to carnivorous plankton as copepods, siphonophores and *Sagitta*. Qasim (1970) estimated the death and decay of a *T. erythraeum* bloom west of Cochin, India resulted in the release of 1.4-2.2 μgN·[m^3·d]$^{-1}$ into surrounding oligotrophic waters. These observations lead Naqvi and colleagues (1986) to conclude that *Trichodesmium* release of phosphate and nitrate could be responsible for the "excess" nitrogen characterizing Red Sea waters relative to inflowing waters from the Gulf of Aden. Karl and co-workers (this volume) have reported 3620 and 583 fold increases in particulate N and P concentrations in *Trichodesmium* blooms north of Hawaii; ammonium levels increased from 0.23 to 1.39-1.5 μM·L^{-1} in dense patches of the alga. Release of N and P from North Atlantic colonies would increase ammonium and orthophosphate levels of Barbados by 2 and 12-fold, respectively (Borstad 1978a,b). Although the decay and lysis of bloom-forming cyanobacteria should be accompanied by large additions of recyclable N and P in other oceanic systems, increases are not always observed (e.g., in the Red Sea following the crash of *Trichodesmium* in 1978; Shaikh et al. 1986).

3. *Nodularia-Aphanizomenon*

Like the *Trichodesmium*-dominated tropical North Atlantic, Pacific, Indian Oceans as well as the Red and Caribbean Seas, surface Baltic waters are dominated by planktonic cyanobacteria, specifically a *Nodularia spumigena-Aphanizomenon flos-aquae* assemblage, visible as summer surface aggregations since late in the last century (Horstmann 1975; Edler 1979; Hobro 1979; Kononen and Niemi 1984; Smayda 1989). Blooms of this assemblage have been attributed to elevated phosphorus concentrations from local runoff into the warm, stable summer water column even back to 1884 when local P inputs probably accounted for the blooms observed by Prince Albert of Monaco (Horstmann 1975). As will be discussed, the fate of the Baltic summer cyanobacteria blooms follows many of the same paths identified for *Trichodesmium* in the oceanic systems except for the absence of a specific macrozooplankton grazer mimicking *Macrosetella*'s role in *Trichodesmium* colonies of more southerly climes. This minimal zooplankton demand favors even greater dependence on bacterial-microbial loop dynamics for bloom disappearance than estimated for oceanic *Trichodesmium* communities.

Nodularia-Aphanizomenon blooms are responsible for large inputs of fixed N in the Baltic, providing 10^5 tons in 1974, equivalent to total N runoff from the land (Rinne et al. 1981). In Bothnian Bay and Sea, N-fixation was responsible for 22 and 1500-5000 tons, respectively, or $<0.1\%$ and 5-15%, respectively, of total input to these systems. More recently, Larsson et al. (1985) estimated total N-fixation in the Baltic at 130,000 tons annually.

Nodularia first appears in the water column as single, straight trichomes with all cells actively photosynthesizing and respiring; mucilage production is minimal and bacterial colonization is low (Bursa 1968; Hoppe 1981). Gradually, trichomes align and mucilage and bacteria are observed on the trichomes; sections of trichomes cease metabolizing and typical cell morphology is altered. The aligned trichomes are initially colonized by marine fungi (*Chytridium*), only to disappear as bacteria become the dominant colonizers. Bacterial succession proceeds from small cocci and colonies at foci of infection on the trichomes and rods evenly distributed about the filaments. The attachment can be focused at heterocysts (as Paerl 1978 has described) but not always; the uptake of ^{3}H-amino acids occurs in bacteria coating most of the trichomes (Hoppe 1978, 1981). Massive agglomeration of *Nodularia* trichomes follows and bacteria proliferate (often forming 2-3 layers) with concomitant increases in microbial grazers including amoeba, ciliates, flagellates and rotifers; crustacean larvae are also present; stalked diatoms and flagellates also abound (Bursa 1968; Hoppe 1981). As the *Nodularia* aggregates age, the filamentous bacterium *Nocardia* increases in number and induces lysis in *Nodularia* cells. Increasing lysis coupled with increasing weight of the attached colonizers results in sedimentation of some portions of the bloom (Hoppe 1981). Very old material is typified by large numbers of akinetes, a matrix of amorphous material and a network of structural elements, former *Nodularia* filaments.

Nodularia blooms can provide dissolved organic phosphorus and carbon for the surrounding microheterotrophs. For example, in a 6 d study in the open Baltic, an increase in cyanobacterial biomass over the last 3 d (15.4-27.5 $\mu gC \cdot L^{-1}$) was accompanied by increases in DOC from 396 to 456 $\mu gC \cdot L^{-1}$ (Wulff and Cederwall 1984). These represent net increases in organic pools, however; total supplies should be much larger.

Bacteria in *Nodularia* blooms receive considerable utilizable substrate from the release of photosynthetic products from the colonies as well as the excretion of DOM from the microbial community in the colonies. Hoppe (1981) has measured DO^{14}C production in ageing *Nodularia* blooms. Release rates from young flocs approximated 11-39% of total carbon fixation resulting in 3-17% of primary production going to the colony bacteria. Older but still active colonies had release rates of 26% with a similar transfer (17%) of primary production to the bacteria. Old flocs, with declining carbon fixation rates and large numbers of inactive or decaying cells,

released 22-57% of the carbon fixed with bacteria consuming 8-44% of primary production from the colony. Assuming carbon fixation rates in the bloom approximated 445 $mgC \cdot [m^2 \cdot d]^{-1}$ (Wulff and Cederwall 1984) and only one-half this rate for late stage colonies, release in the three stages of the *Nodularia* bloom would represent 49-174, 116 and 49-127 $mgC \cdot [m^2 \cdot d]^{-1}$, respectively. Soluble production supported a total bacterial biomass of 30-61 mg in 2 L of *Nodularia*, a bacterial biomass normally observed in 3-6 m^3 of water (Hoppe 1981).

Two short-term studies off the southwest coast of Finland in 1981 indicated that bacterial production and mineralization of cyanobacterial production could be substantial in summer Baltic waters (Kuparinen 1984; Tamminen et al. 1984). Early in the bloom, bacterial production formed about 9.6% of apparent net primary production; however, as the bloom aged, bacterial heterotrophy exceeded autotrophy in the bloom by 2.7 fold. Tamminen and colleagues (1984) suggested that low molecular weight extracellular products supported bacterial production early in the bloom while larger compounds from cell lysis in the decomposing bloom were the primary substrates for the bacteria late as the bloom ages. Further support for lysis during decomposition of surface *Nodularia* blooms was also reported by Gabrielson and Hamel (1985); soluble organic N and P increased in old *Nodularia* populations, potentially supporting the active microbial loop even after autotrophic carbon fixation had ceased.

3.2. *NODULARIA-APHANIZOMENON* INTERACTIONS WITH ZOOPLANKTON

In contrast to the strong autotroph-herbivore linkage in tropical *Trichodesmium-Macrosetella*, no crustacean grazer has been identified as a major consumer of Baltic cyanobacteria blooms. Heerkloss et al. (1984b) reported low assimilation efficiencies ($\sim$40%) for the copepod *Acartia tonsa* when the cyanobacteria *Oscillatoria* and *Nodularia* dominated inshore waters of the Darss-Zingst estuary. In another sub-estuary of the Baltic, grazing on cyanobacteria removed only 3% of the biomass while 10% of eucaryote biomass was consumed during the diatom-chlorophyte spring bloom (Heerkloss et al. 1984a). The summer cyanobacteria bloom also accompanied the lowest zooplankton biomass for the year. Hernroth and Ackerfors (1979 in Forsskåhl et al. 1982) note that cyanobacteria common to the Baltic in July-August do not provide food for any zooplankton group. Wulff and Cederwall (1984) assumed minimal zooplankton grazing in a *Nodularia spumigena* bloom in the open Baltic in 1982 and this premise is repeated throughout literature for the region. Bursa (1968) and Hoppe (1981) both concluded that potential herbivores in *Nodularia* aggregates excluded *Nodularia* cells as potential food items, selecting bacteria and other members of the microbial community of the colony as substrate. Rotifers might exert some grazing pressure on Baltic blooms as Horstmann (1975) reported that *Keratella quadrata* and *K. cochlearis recurvispina* bit single cells from trichome bundles of *Aphanizomenon flos-aquae*; however, in other bloom-forming cyanobacteria, rotifers rarely exert substantial grazing pressure (e.g., Sellner et al. in prep.). Except for one report of zooplankton-mediated demise of summer *Nodularia* bloom in the eastern Baltic in the late 1970's (U. Horstmann, pers. commun.), zooplankton grazing appears to remove little cyanobacterial production in the northern latitude fjord.

3.3. *NODULARIA-APHANIZOMENON* INTERACTIONS WITH OTHER GRAZERS

No references were found indicating grazing of Baltic cyanobacterial blooms by higher trophic levels. Schulz and Kaiser (1986) report increasing stocks of the Baltic planktivores herring and sprat and attribute the increase to increasing phytoplankton levels in the system; however, the increase could not be directly attributed to cyanobacteria increases in the late summer. Similar increases in benthic macrofauna biomass above the halocline (Cederwall and Elmgren 1980) have been attributed to general eutrophication in the Baltic.

3.4. SEDIMENTATION OF *NODULARIA-APHANIZOMENON*

Several researchers have suggested that colonization of surface blooms ultimately results in cell lysis and increasing weight of the bloom community, exceeding buoyancy provided by cell gas vacuoles, leading to sedimentation (Bursa 1968; Hoppe 1981). However, *Nodularia* and *Aphanizomenon* have not been reported in sediments of the Baltic and only rarely in sediment trap collected material from the region (Forsskåhl et al. 1982; see below) and the overwhelming consensus that cyanobacteria blooms decompose as floating algal-microbial biotopes supports minimal sedimentation. Walsby (this volume) reports that *Nodularia* and *Aphanizomemon* require 8 and 5 bars pressure, respectively, to collapse gas vacuoles and therefore, with the relatively shallow depths of the Baltic, sedimentation to the bottom is unlikely. However, some portion of the phytoplankton assemblage in the bloom might be sinking out; associated eucaryotes can certainly settle out while 34% of decomposing *Nodularia* is refractory (Gabrielson and Hamel 1985) indicating that older bloom detritus might be making it to depth. Sörensson and Sahlsten (1987) reported a small peak in ATP at the thermocline during a bloom of *Nodularia spumigena* in summer, 1982 and proposed that the alga might be decomposing at the surface and above the thermocline. Further, using data from Cederwall (1987 in Sörensson and Sahlsten 1987), total carbon lost from a Baltic surface cyanobacteria bloom in July-August, 1982 would approximate 3% of bloom carbon. Gundersen (1981) stated that dead cyanobacteria from a surface bloom sank rapidly through the water column to collect at the pycnocline where rapid remineralization occurred, accompanied by nitrification and denitrification and no accumulation of inorganic N species in the system. *Nodularia* and *Aphanizomenon* have been collected at 220 m in the central Baltic Sea in 1989 (R. Boje, pers. commun.). Kononen and Nômmann (this volume) reported storm-induced dispersal of *Nodularia* through the water column in the Gulf of Finland. In general, however, sedimentation of bloom cyanobacteria is low (Kuparinen et al. 1984) with N from decomposing organic matter passing rapidly into solution (e.g., Forsskåhl et al. 1982).

Changes in cell carbon to nitrogen ratios and other environmental stresses can induce akinete formation in *Nodularia* (Huber 1984); these negatively buoyant resting stages rapidly sink to surface sediments for seeding new blooms on advent of warm, stable, P-rich conditions in following summers. In western Australia, akinete densities have increased 20 and 100 fold over 4 years in two estuaries typified by summer *Nodularia* blooms. Akinetes remain viable for years and accumulation over time guarantees recurring summer blooms as long as phosphorus is available in warm, calm summer months. With repetitive *Nodularia* blooms in summer Baltic waters, one can presume that densities of *Nodularia* akinetes in surficial sediments of the Baltic must be high.

3.5. *NODULARIA-APHANIZOMENON* BLOOMS AND NUTRIENT RECYCLING

Decomposition of Baltic *Nodularia* blooms is also accompanied by temporary increases in inorganic nutrients, as noted for coastal India and Red Sea *Trichodesmium* blooms. Gundersen (1981) reports that the massive die off of cyanobacteria in August was accompanied by a sudden increase in ammonium at the pycnocline followed by an increase in nitrate in the water column. However, denitrification rapidly consumed the available nitrate and N originally fixed by the cyanobacteria was lost from the system. Huber (1986) noted that following a *Nodularia* bloom in the Peel-Harvey estuary in Australia, organic nitrogen declined, ammonium increased followed by increases in nitrate plus nitrite. Eighty percent of a cyanobacteria bloom was remineralized in the water column off Finland in 1980 (Kuparinen et al. 1984). Lindahl and co-workers (1980) and Schulz and Kaiser (1986) proposed that nitrogen fixed by *Nodularia* blooms is primarily liberated when the bloom decomposed in the surface layer. Horstmann (1975) noted dead floating trichomes of *Nodularia* and *Aphanizomenon* released nutrients during decomposition, probably

arising from photolytic destruction of chlorophyll in surface material, giving rise to a whitish-yellow bloom appearance over time. Gabrielson and Hamel (1985) noted that rapidly decomposing *Nodularia* released both organic and inorganic N and P (87% of total N and P over 70 d), potentially supporting phytoplankton (dinoflagellates and diatoms) typical of fall Baltic waters (Edler 1979; Hobro 1979). These observations are not surprising considering the overwhelming belief that the fate of Baltic cyanobacteria blooms is decay and decomposition rather than incorporation into biomass of higher trophic levels (Bursa 1968; Horstmann 1975; Niemi 1979; Lindahl et al. 1980; Gundersen 1981; Hoppe 1981; Forsskåhl et al. 1982; Sörensson and Sahlsten 1987).

4. General Overview of the Fate of Marine Cyanobacteria Blooms

Trichodesmium and *Nodularia-Aphanizomenon* blooms in the tropical and sub-polar regions of the world's oceans act as large concentrators of organic carbon, nitrogen and phosphorus for subsequent recycling through an active microbial loop maintained within the colonies of the blooms. As nitrogen fixers in oligotrophic environments, the algae fix "new" nitrogen into cellular pools for subsequent remineralization and supply for continued production of other phytoplankton in these oligotrophic regions. The quantities of nitrogen added to these environments, and recycled primarily through the microbial loop, total 4.8 Tg and 10^5 tons for the Caribbean and Baltic Seas, respectively (Rinne et al. 1981; Capone and Carpenter 1982). Nitrogen and carbon fixed by *Trichodesmium* is not supporting production in trophic levels any higher than copepods (and perhaps a few fish species) (see Fig. 1) while summer Baltic cyanobacteria populations supply C, N and P to the active microbial loop of the colonies and perhaps of the pycnocline/thermocline (Fig. 2).

The shunting of the majority of bloom production to the microbial loop for both bloom taxa is not unlike fates for freshwater cyanobacterial blooms (Reynolds 1984). Generally, macrozooplankton and nekton are minor grazers on the bloom while very active microbial communities typify bloom areas (see reviews by Paerl 1988, 1991). These communities may represent a "sink" for bloom production as intensive grazing on microzooplankton in bloom colonies by predators outside of the cyanobacteria communities has rarely been observed. As a result, carbon may be intensively cycled in the microbial community (spinning wheel) and respired to CO_2. Nitrogen and phosphorus should be remineralized through excretion, sloppy feeding and death in the microbial community, providing regenerated N and P for phytoplankton productivity in the region. To this end, Wulff (1988) and Wulff and colleagues (1986) have estimated that 73-90% of the N assimilated in the summer is recycled in the Baltic, primarily in the surface mixed layer.

With the general qualitative nature of data for these two bloom formers, future research programs might wish to focus on quantifying the flows of carbon, nitrogen and phosphorus arising from the following considerations.

1) Bacteria are the driving force in carbon and nutrient dynamics in marine cyanobacteria blooms. Future research should focus on estimating the bacterial biomass and productivity in colonies as well as production rates and quality of low molecular weight photosynthates and the carboxylated polysaccharide mucilage of the trichomes and colonies. This latter DOM pool should be the largest organic carbon source and could provide a long-term substrate reservoir for colony bacteria and fungi; its rate of production and subsequent hydrolysis and consumption would be critical to determining carbon supply to the bacterioplankton.

2) Since researchers studying *Trichodesmium* and *Nodularia* blooms indicate that decomposition/decay are the likely fates for these cyanobacterial accumulations, simple but classic

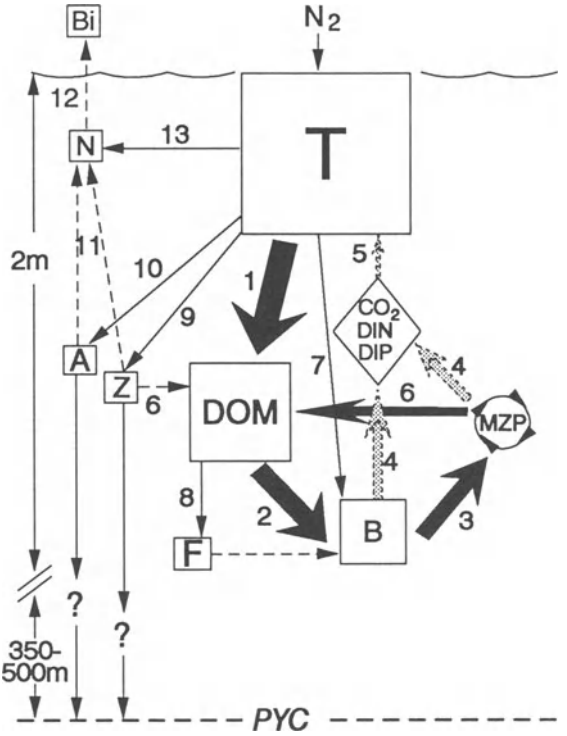

Figure 1. Conceptual diagram of carbon and nutrient cycling in *Trichodesmium* blooms. Initially, the colony and epibiota act as a mutualistic assemblage followed by a decomposition phase. Thickness of the arrows indicates magnitude of the transfer while box size is the relative size of the carbon and nutrient pool. The dominant fate is (1) the production of DOM through (a) exudation of products from photosynthesis and N-fixation, (b) mucilage production and (c) leakage of material from dying and dead cells. This material is the primary substrate (2) for heterotrophic bacteria of the colony. Bacteria are the primary prey (3) for the complex microheterotrophic community of protozoa, flagellates, ciliates, hydromedusae and fish and polychaete larvae. Metabolism in the bacteria and microzooplankton (Microbial Loop) yields respired CO_2 and inorganic nutrients (4) for continued autotrophy (5) while the microzooplankton produce DOM (6) from sloppy feeding, excretion and mortality. Bacteria can also produce exoenzymes (7) for the breakdown of *Trichodesmium* mucilage and disintegrating cells. Less important consumers include fungi feeding on mucilage (8). Mesozooplankton including the harpacticoid copepod, *Macrosetella gracilis*, and salps can also be significant herbivores (9) as well as pelagic tunicates (10), with subsequent ingestion by higher trophic levels (11) including birds (12). Planktonic grazer impact is considered small because of the persistence of *Trichodesmium* blooms even with growth rates of several weeks to months. Grazing pressure by larger pelagic invertebrates, e.g., Sargassum crab, and nekton (13), remains uncertain. The symbol code is T=*Trichodesmium*, DOM=dissolved organic matter, B=bacteria, F=fungi, MZP=microzooplankton, Z=mesozooplankton, A=appendicularians, N=nekton and pelagic macroinvertebrates, Bi=birds and PYC=pycnocline. CO_2, DIN and DIP represent carbon dioxide and inorganic nitrogen and phosphorus.

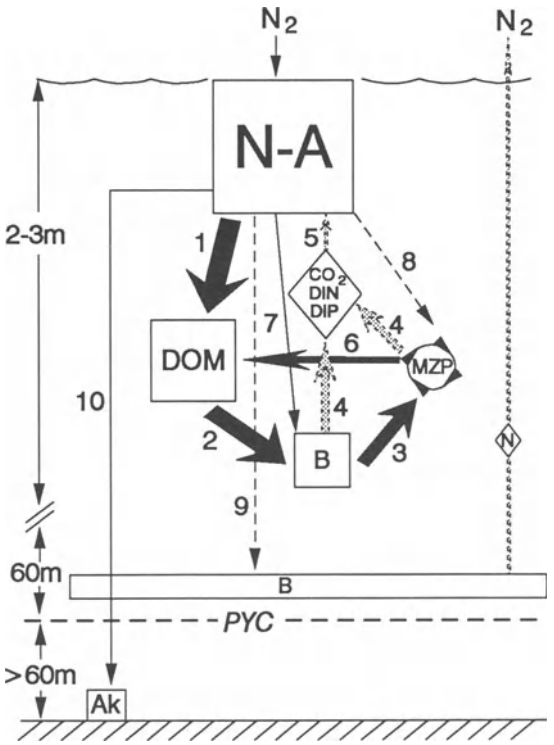

Figure 2. Conceptual diagram of carbon and nutrient cycling in *Nodularia-Aphanizomenon* bloom. As in *Trichodesmium* blooms, a DOM-bacteria-microzooplankton-recycling food web is active, primarily as a decomposition cycle. As depicted for *Trichodesmium* (Fig. 1), carbon and nutrient transfers 1-7 are dominant in these Baltic and Australian blooms. MZP change in the brackish coastal blooms to include rotifers, trivial grazers (8) on the bloom. However, in contrast to the tropical cyanobacterium blooms, there are no crustacean or larger metazoan grazers. A small fraction (0-3%) of the *Nodularia-Aphanizomenon* bloom might sink to the pycnocline (9) where it is remineralized and denitrified. *Nodularia* also forms akinetes (Ak) as resting stages that are negatively buoyant and sink rapidly to the sediments (10). Arrow thickness, box size and symbols as in Fig. 1.

decomposition studies should be undertaken perhaps in larger mesocosms so that bottle-induced mortalities (Mague et al. 1974; McCarthy and Carpenter 1979) might be reduced and sedimentation, if it occurs, could be quantified.

3) Do surface blooms rich in gas vacuolated forms ever sink out? Borstad and Brinckmann-Voss (1979), Bursa (1968) and Hoppe (1981) all argue for sedimentation as a function of increasing weights of associated epi-flora and -fauna. Kononen and Nômmann (this volume) present convincing evidence for storm-induced mixing to depth. However, there is little evidence from trap-collected materials that cyanobacteria sink through the water column or make it to the benthos. Species composition or pigment signatures (e.g., zeaxanthin) in size-fractionated

submersible-collected particulates or in particulates from sediment traps deployed in the North Atlantic and Baltic might provide needed differentiation of *Trichodesmium* and *Nodularia* contributions to the deeper water column or benthic heterotrophs. In addition, akinete densities in declining blooms, traps and surficial sediments of the Baltic could be estimated for indicating re-inoculation potential for recurring blooms.

4) Böttger-Schnack and Schnack (1989) have noted large accumulations of oil droplets in *Macrosetella* from the Red Sea. Analysis of lipid distributions in the copepods, *Trichodesmium* and offspring as well as other potential grazers (e.g., Kimor and Golandsky's tintinnids) might aid in determining the fraction's of each animal's diet that the cyanobacteria might fill. Accessory pigments (e.g., zeaxanthin) and their degradation products might also be used in the food web dissection.

5) The role of the highest predators, nekton and larger invertebrates, in processing *Trichodesmium* production remains confusing, and nothing is reported for non-planktonic metazoan consumption of the Baltic blooms. Planktivorous fishes in spatially dilute particulate fields should find *Trichodesmium* and *Nodularia-Aphanizomenon* aggregations ideal substrates for direct consumption of autotrophic carbon as well as the large mass of heterotrophic biomass included in the colonies. Yet nekton consumption remains questionable. Are blooms too dense to be processed or, alternatively, cause asphyxiation in nektonic predators? Large mesocosms might provide some answers on the relative importance or inhibitory effects of marine cyanobacteria blooms on these grazers.

6) Rapid disappearance of cyanobacteria blooms typifies some systems; dense surface aggregations are present one day then disappear overnite without passage of any mixing event (Borstad 1982). Is this catastrophic decline attributable to viral infestation and lysogeny? Densities of viruses range from 10^7-10^{11}·ml^{-1} *in situ* (Proctor and Fuhrman 1990) and have been shown to infect and lyse bacteria, coccoid and filamentous cyanobacteria, diatoms and cryptophytes (Safferman and Morris 1964; Bratbak et al. 1990; Klut and Stockner 1990; Suttle et al. 1990). With cyanobacteria blooms providing high probabilities for encounter between host and phage, viral infestation could be a major factor in instantaneous lysis of some trichomes in a colony or complete blooms in a system.

7) Do storm-induced mixing events permanently remove surface cyanobacteria to aphotic and catabolically-dominant depths or do vacuolated taxa simply rise to the surface to reappear as blooms? Certainly, bloom-forming cyanobacteria are found at depth; are these simply populations mixed to depth through storm activity or are they cells that have settled out due to diurnal accumulation of carbohydrate ballast (Kromkamp and Walsby this volume), only to ascend to the surface due to the strong gas vacuoles present? Should we consider vertically distributed trichomes simply intermediate stages of a dynamic surface bloom occasionally mixed to depth through meteorological forcing or intracellular density increases?

There is much to be done in the next decade and the interest and techniques are available and expanding in the research community. As noted for bloom-forming taxa of other aquatic systems, we are only beginning to comprehend the importance of blooms in carbon and nutrient cycling in our coastal and open oceans; much excites and awaits us.

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SPATIO-TEMPORAL DYNAMICS OF THE CYANOBACTERIAL BLOOMS IN THE GULF OF FINLAND, BALTIC SEA

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ABSTRACT. High-resolution on-line measurements of chlorophyll *a*, particles, salinity and temperature supplemented with stationary vertical casts of CTD, nutrients and phytoplankton, from August 1990 were used to investigate the spatio-temporal patterns of summer cyanobacterial blooms along the axis of the Gulf of Finland, the Baltic Sea. These measurements were supplemented with information about phytoplankton species composition and the vertical distributions of chlorophyll and nutrients while the ship was stationary. The results showed that the Gulf of Finland is divided into several sub-basins which are separated from each other by quasi-stationary fronts related to the bottom topography and circulation patterns of the Gulf. The basins and fronts had their own characteristic bloom dynamics - wind-induced external energy inputs controlled bloom formation within the sub-basins, whereas the vertical, front-related transport processes had an additional impact on the bloom in frontal areas. Nutrient reserves and inorganic N:P ratios below the seasonal thermocline differed between sub-areas.

1. Introduction

Summer cyanobacterial blooms, consisting mainly of the filamentous, nitrogen-fixing species, *Aphanizomenon flos-aquae*, *Nodularia spumigena* and *Anabaena* spp., are regular phenomena in the Baltic Sea (e.g. Öström, 1976, Niemistö et al. 1989). The bloom-forming species are toxic or potentially toxic (Sivonen et al. 1989). The blooms usually develop during the warmest season when the recreational use of the near-shore waters is maximal. These bloom events are therefore subject of considerable public interest.

The summer mass-development of cyanobacteria is characterized by several features fundamentally different from the phytoplankton vernal blooms. First of all, the bloom begins at the time of strongest seasonal thermal stratification. The vertical fluxes of both dissolved and particulate inorganic and organic material are apparently regulated by the introduction of external energy sensu Margalef (1978) (mainly turbulence generated and spread from the surface and/or upwelling). Secondly, the spring bloom, as shown in field and experimental investigations, is limited in most of the Baltic Sea areas by nitrogen (Tamminen 1990, Graneli et al., 1990). The bloom-forming cyanobacteria are biologically unique in being able to fix dissolved molecular nitrogen. Nitrogen-fixation is estimated to be account for ca. 10 % of the total nitrogen input into the Baltic Sea (Rönner 1985, review by Leppänen et al. 1988). In contrast to other taxonomic groups, the nitrogen-fixing cyanobacteria are able to utilize free, dissolved phosphates in the absence of dissolved nitrogen. Thus phosphorus limitation prevails during the blooming of the nitrogen-fixing cyanobacteria.

The heavy summer blooms of the cyanobacteria in the open Baltic Proper, Gulf of Finland and Gulf of Bothnia have been related to vertical exchange processes that bring up Baltic deep waters with a low nitrogen to phosphorus ratio (Niemistö et al. 1989). Both field and experimental studies show that blooms of *Aphanizomenon flos-aquae* are associated with phosphorus inputs (Niemi 1988, Wallström 1988, unpublished data of the Finnish Institute of Marine Research). The blooming of the

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toxic species *Nodularia spumigena* is regular in the Baltic Proper, but more irregular in the Gulf of Finland (Kononen and Niemi 1986, Niemistö et al. 1989, Viljamaa 1988), and seems to be controlled by the upper layer temperature. The germination of its akinetes requires temperature of at least 16 °C (Huber 1984). Occasionally, blooms of other, more opportunistic cyanobacteria may also be associated with specific meteorological and hydrodynamic conditions. This was the case in autumn 1987, for example, when a freshwater cyanobacteria, *Microcystis aeruginosa*, formed a heavy bloom in the eastern Gulf of Finland (Niemi 1988). A more detailed study of the factors triggering and controlling bloom initiation and development, taking into account the hydrodynamics is, however, lacking.

The need to measure distributions of physical, chemical and biological properties over large areas synoptically and over sufficiently long time periods has been recognized in several oceanographic disciplines. Compared to the oceans, the dominant time scales of the weather pattern and related hydrodynamics are much shorter in the Baltic Sea. This makes it very difficult to carry out investigations of their influence on the biological components of the system using traditional monitoring methods. The aim of the present study was to investigate cyanobacterial blooms in the Gulf of Finland over different spatio-temporal scales ranging from basin-wide to mesoscales that are typically not resolved at the same time.

2. Study Area

The hydrographic conditions of the stratified non-tidal Baltic Sea are unique and not easily compared to any oceanic or lake aquatic system. The Baltic Sea consists of a series of basins, of which the Gulf of Finland is the easternmost water body (Fig. 1). The Gulf of Finland is a channel-shaped (500x80 km), transition area running in an east-west direction between real brackish water in the Baltic Proper (salinity 6-7 ‰) and the almost limnic area outside Leningrad. It has a relatively high mean river run-off from the surrounding drainage area (mainly the River Neva in the east) compared to the Baltic Proper (Baltic Sea Environment Protection Commission 1986), which is reflected as profound changes in the salinity structure along the central axis of the Gulf. A complicated coastline comprising a multitude of bays, peninsulas and islands, combined with the related bottom topography, has an additional impact on the heterogeneity of water properties in the Gulf of Finland.

Owing to the long stagnation period of water exchange between the Baltic Sea and the North Sea, the Baltic deep water salinity has decreased continuously since the late 1960s (Baltic Sea Environment Protection Commission 1990, Matthäus 1990). As a result of this development and the bottom topography of the Gulf of Finland (the depth gradually decreases from 100 m in the west to 20 m in the eastern open parts), the primary halocline has extended only to the western part of the study area since the early 1970s. Anoxic bottom water has been reported only twice since the early 1970s: in 1980 and 1982 (Andersin and Sandler 1991). The main nutrient input (both phosphorus and nitrogen) in the eastern part is of direct anthropogenic origin due to the River Neva, which accounts for almost 80 % of the total inputs from the whole drainage area of the Gulf of Finland (Nehring 1990, Baltic Sea Environment Protection Commission 1987).

3. Research Strategy And Methods

Spatio-temporal developments in the pelagic ecosystem of the Gulf of Finland were studied by means of measurements made onboard RV Aranda (Finland) along longitudinal sections running

down the central axis of the Gulf during August 3-14, 1990. Semi-continuous measurements were performed using a flow-through system during all the transects. In addition, two transects were covered with vertical samples taken at fixed stations (Table 1).

TABLE 1. Longitudinal transects along the axis of the Gulf of Finland and the parameters measured.(S‰= salinity, T °C = temperature, chl *a* = chlorophyll *a*). Phytoplankton samples were taken from the flow-through water with lower frequency than the other measurements were made. From the stations phytoplankton samples were taken only from 5 m.

Transect	Date	Continuous measurements at depth of 5 m					Vertical measurements at stations			
no	(1990)	S ‰	T °C	chl *a*	phyto-plankton	particles	CTD	phyto-plankton	chl *a*	nutrients
I	3.-4.8.	x	x	x		x	x			
II	4.-5.8.	x	x	x		x	x	(x)	x	x
III	6.8.	x	x	x		x	x			
IV	7.8.	x	x	x	(x)	x	x			
V	9.-10.8	x	x	x		x	x			
VI	10.-12.8.	x	x	x		x	x	(x)	x	x
VII	13.-14.8.	x	x	x		x	x			

3.1. CONTINUOUS MEASUREMENTS WITH THE FLOW-THROUGH SYSTEM

Shipboard field data were obtained quickly and periodically as the ship was steaming along the central axis of the Gulf of Finland. The duration of each large-scale transect, typically one to two days, ensured a semi-synoptic approach (Table 1). The measurements of particles, chlorophyll *in vivo* fluorescence, temperature and conductivity were obtained by pumping the seawater from a depth of 5 m up to the sensors. The particle concentration was determined using an on-line particle size analyzer (PMT 6000), which counted particles with an equivalent spherical diameter ranging from 1 to 2000 μm in 32 size classes. During the transect surveys the ship speed was held at about 10 knots, giving a spatial resolution of about 300 m. *In vivo* fluorescence of the chlorophyll pigments was measured with a Variosence III fluorometer in the flow-through tank. Information about vertical distributions from different depths while the ship was stationary was obtained using a Neil Brown Mark V CTD sonde equipped with a rosette sampler.

3.2. ANALYTICAL METHODS

Chemical and biological measurements were made twice, during transects II (August 3-4) and VI (August 11-12), at 12 stations located along the axis at ca. 30 nmi intervals (Fig. 1). Measurements were made during a longer period (July 12 to August 17) at station LL6, altogether 10 times. These results are presented and discussed in more detail by Kononen et al. (in prep.).

Nutrient samples were taken with a rosette sampler from depths of 0, 5, 10, 15, 20 and 30 m. The dissolved inorganic nitrogen (-NO_3, -NO_2 and -NH_4) and phosphorus (-PO_4), as well as total nutrients, were analysed according to the Guidelines for the Baltic Monitoring Programme for the

Second Stage (Baltic Marine Environment Protection Commission 1983). Particulate phosphorus and nitrogen were analysed from material filtered out with Whatman GF/C glass fiber filters as described by Laakkonen et al. (1981). The filters were prerinsed with sterilized, filtered deionized water before filtering the sample. The nutrient values from the discrete depths were integrated from the surface to the beginning of the thermocline to represent the total nutrient store in the upper mixed layer.

Chlorophyll *a* samples were taken from the same depths as the nutrient samples. However, additional samples were taken at depths of 2.5 and 7.5 m for chlorophyll *a* analysis. The chlorophyll *a* concentration was determined from material filtered out with Whatman GF/C glass fiber filters and extracted in 96 % ethanol and measured fluorometrically on a Perkin Elmer LS-2B fluorometer according to Baltic Sea Environment Protection Commission (1983).

Phytoplankton samples were taken from a of depth 5 m. Additional samples were taken from the flow-through water during transect IV (August 7-8) The samples were preserved with Keefe-solution (Keefe 1926) which is known to be a suitable preserver especially for cyanobacteria with gas vacuoles (Hällfors et al. 1979). The dominating phytoplankton taxa were identified from net samples during the cruise, and these species later counted from quantitative samples using the Utermöhl technique.

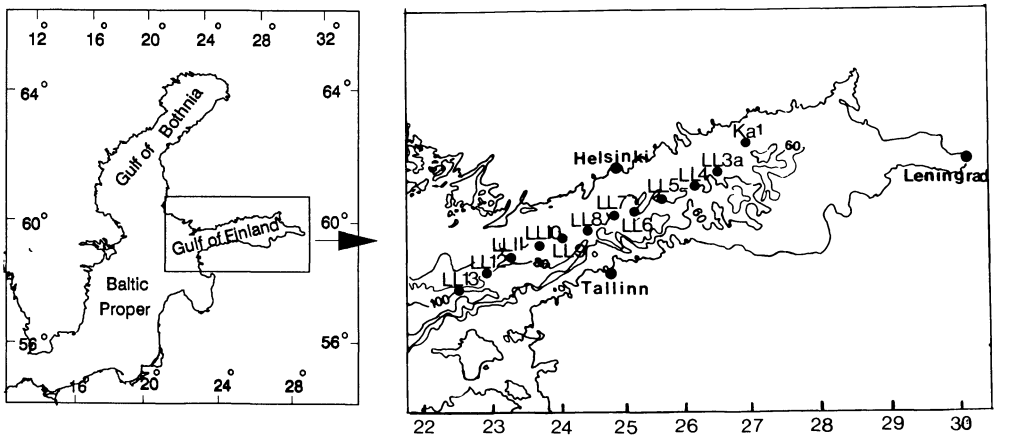

Figure 1. Study area and the location of the fixed sampling stations and the longitudinal transects along the axis of the Gulf of Finland.

4. Results

4.1. METEOROLOGICAL CONDITIONS

The weather pattern during the study period can be depicted as several sunny and calm periods with typical temporal scales of about a week, interrupted by the passage of atmospheric fronts and related stronger wind events.

At the beginning of the study period, in the middle of July, the Gulf of Finland was influenced by a low pressure area located in northern Scandinavia. During July 15-16 the low pressure area and

adjacent atmospheric fronts moved over the Gulf of Finland producing rain and northerly winds, with maximum speed reaching 15 ms^{-1} (Fig. 2). The low pressure centre remained over the Baltic countries and northern Soviet Union, gradually weakening up until July 24. This period was characterized in the middle of the Gulf of Finland by diminishing wind speeds and gradually increasing solar radiation from 5.3 MJ m^{-2} on July 15 to 24.7 MJ m^{-2} on July 29.

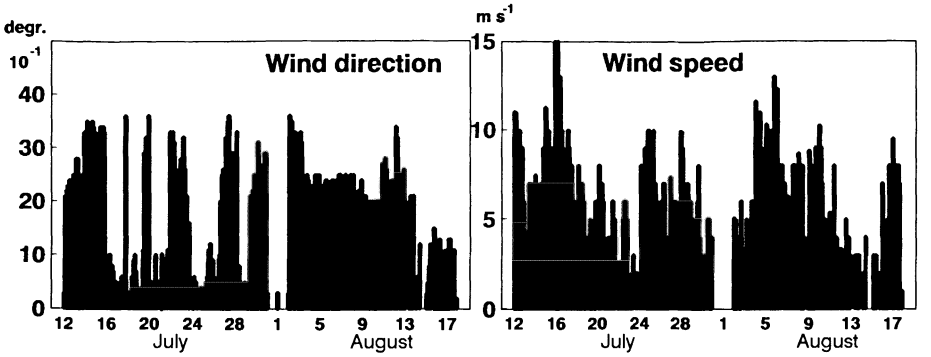

Figure 2. Wind speed and direction at Bågaskär Island, situated in the middle of the Gulf of Finland, off Helsinki.

Thereafter the study area was influenced by a high pressure area moving from northern Scandinavia to central Europe. The end of July and the beginning of August were characterized by calm weather and high solar radiation caused by the high pressure areas moving over central Europe. From August 4 onwards an atmospheric front followed by a low pressure area again moved over the Gulf of Finland. South-westerly winds dominated and the wind speed rose to 6-10 ms^{-1} throughout the study area. The area remained sandwiched between two atmospheric fronts associated with low pressure areas up until August 9 when the overpassage of a front again caused increasing SW-winds and lower solar radiation. The atmospheric fronts thereafter remained outside the area and a high pressure area developed over the Baltic countries and northern Finland. Rather calm, sunny weather prevailed up until August 18 when a cold front causing increasing wind speed again passed over the Gulf of Finland.

4.2. LARGE-SCALE SPATIO-TEMPORAL DISTRIBUTIONS

4.2.1. Salinity And Temperature (Water-Masses). By the beginning of the study period the upper layer temperature had reached typical late-summer values in the Gulf of Finland (about 17-18 °C) and the strong seasonal thermocline had developed beneath the uppermost mixed layer at about 10 m depth (Fig. 3b). The upper layer salinity ranged from 6.0 ‰ at the entrance to the Gulf of Finland to 3.5 ‰ near Suursaari island in the east (Fig. 4 a).

At least three, quasi-stationary frontal systems were distinguished. The frontal systems were well evident in the vertical salinity and temperature sections (Fig. 3), as well as in the continuous

measurements made in the surface layer along the axis of the Gulf of Finland (Fig. 4). The fronts were related to the bottom topography and the general circulation pattern in the Gulf of Finland. Generally, the large-scale salinity and temperature patterns were inversely correlated. The water in frontal areas had relatively the lowest temperature, indicating vertical fluxes (frontal upwellings). A more detailed description of these fronts, their persistence, intensity and location as well as mesoscale dynamics, will be given by Nõmmann et al. (in prep.).

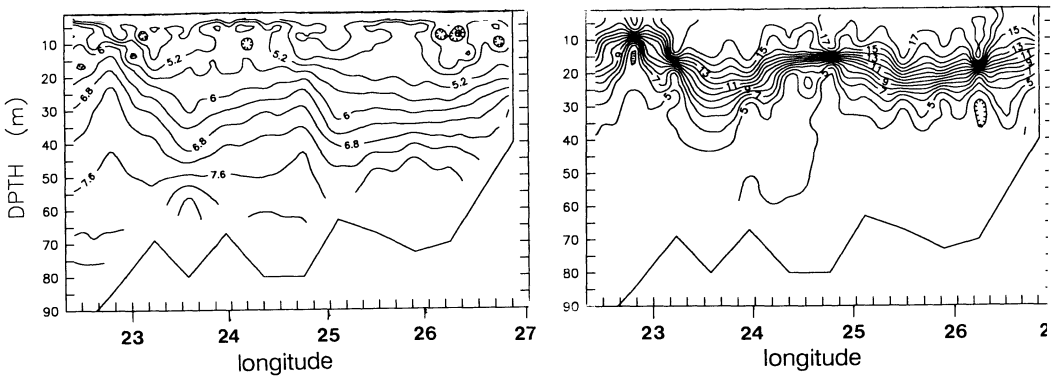

Figure 3. Isopleths of a) salinity and b) temperature along the central axis of the Gulf of Finland on 6.8.1990.

A low salinity water filament, apparently of coastal origin, was observed during August 3-5 at about 24°50′, but was no longer evident at a later date (Fig. 4a).

Although the large-scale pattern of individual water masses separated by fronts was persistent in time, the westerly wind event from August 4 onwards triggered, in the western Gulf of Finland especially, vigorous mixing in the upper layer which resulted in the deepening and cooling of the upper mixed layer at some stations. The upper layers also became more saline. (Fig. 4a).

4.2.2. Nutrients. The distribution patterns of all measured nutrients followed the large-scale structure of the water masses. At the beginning of the study period the highest total phosphorus and nitrogen amounts integrated over the upper mixed layer were found at the station situated within the westernmost front and on both sides of the low salinity water mass in the centre of the study area (Table 2). The highest amounts of ammonium (over 2 mmol m^{-2}) were observed at stations located within the basins between the two main fronts (LL11-LL10 and LL6-LL4). Exceptionally high nitrate amounts (2.1 mmol m^{-2}) were observed at station LL6. Phosphate amounts ranged between 0.25-1.35 mmol m^{-2} and the highest value was found at station LL13 located within the westernmost, high-salinity water mass. The particulate N:P ratios were close to the Redfield ratio of 16 at most stations, ranging from 11 at the westernmost station (LL13) to 19 at the frontal station (LL12) and in the east (Ka1).

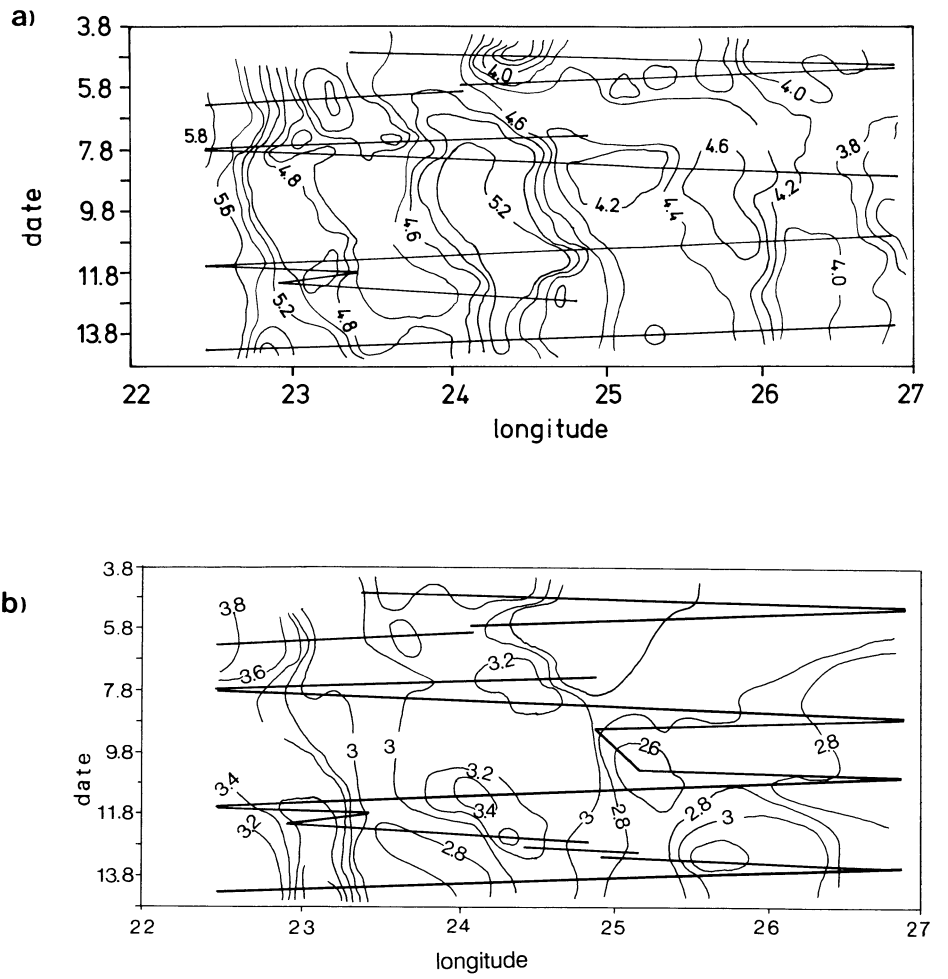

Figure 4. Spatio-temporal distribution of a) salinity (‰) and b) chlorophyll *a* (mg m^{-3}) in the surface layer (5 m) of the Gulf of Finland during 3-13.8.1990. The lines indicate the route taken when steaming back and forth along the central axis of the area.

The inorganic nutrient concentrations and inorganic N:P ratio below the thermocline (depths of 20 and 30 m) were also highly related to the water masses and fronts (Table 3). In the westermost high salinity water (station LL13), especially high phosphate concentrations (over 1.5 mmol m^{-3} in 30 m) and low molecular inorganic nitrogen to phosphorus ratio (1) were observed immediately below the thermocline in 20-30 m. On moving from the western front to the central front towards east the inorganic N:P ratio at 20-30 m gradually increased and was 10 within the western interfrontal

basin (stations LL10-LL9). To the east from the central front the inorganic N:P ratio was between 13 and 17 (stations LL6-LL3a).

The spatial variability of all measured nutrients and nutrient ratios was clearly decreased both in the upper mixed layer and below the thermocline after the wind induced mixing during August 7-8. Phosphorus enrichment of the upper layer was observed in the westernmost end of the transect and both phosphorus and/or nitrogen enrichment within the basin to the east of the central front (stations LL6-LL4). All these nutrient increases were accompanied by an increase in chlorophyll *a*.

TABLE 2. Nutrients (mmol m^{-2}) and chlorophyll a (mg m^{-2}) in the upper mixed layer. Values are integrated over the upper mixed layer according to measurements made at fixed depths. The upper mixed layer depth (UMLD) is determined according to density profiles from a CTD sonde.

Transect II 4.-5.8.1990

Station	UMLD (m)	PO_4	NO_2	NO_3	NH_4	totP	totN	partP	partN	partP: partN	chl *a*
LL13	10	1.35	0.65	0.05	0.00	4.2	230	1.1	13	11	15
LL12	16	0.83	0.80	0.00	0.11	8.6	476	3.1	60	19	54
LL11	11	0.33	0.17	0.22	3.22	7.8	374	2.8	42	14	-
LL10	11	0.25	0.11	0.00	2.31	6.2	303	2.4	40	16	35
LL9	13	0.46	0.33	0.09	0.09	7.5	393	2.8	46	16	48
LL8	10	0.33	0.43	0.75	1.45	6.0	301	3.2	47	15	-
LL7	13	0.50	0.59	0.33	1.14	10.1	588	2.1	36	17	33
LL6	11	0.50	0.57	2.07	3.08	6.5	316	2.8	32	12	27
LL5	11	0.30	0.25	0.00	2.78	5.3	346	3.3	48	14	30
LL4	10	0.25	0.25	0.00	2.00	4.1	320	2.2	36	16	28
LL3a	12	0.56	0.24	0.00	1.14	6.7	349	3.8	60	15	34
Ka1	9	0.90	0.45	0.16	6.80	5.7	246	2.9	56	19	32

Transect VI 10.-13.8.1990

Station	UMLD (m)	PO_4	NO_2	NO_3	NH_4	totP	totN	partP	partN	partP: partN	chl *a*
LL13	10	0.52	0.17	0.00	0.85	5.1	269	2.3	41	18	31
LL12	10	0.35	0.30	0.00	1.65	5.6	363	2.5	44	18	25
LL11	9	0.39	0.15	0.00	2.61	4.4	294	1.7	29	17	26
LL10	11	0.39	0.22	0.00	2.55	4.1	339	2.1	34	16	27
LL9	10	0.33	0.20	0.00	1.43	3.7	245	-	-	-	28
LL8	10	0.40	0.10	0.20	0.90	3.9	240	2.1	37	18	26
LL7	13	0.75	0.13	0.00	1.82	5.2	323	2.0	37	18	32
LL6	11	0.69	0.44	0.00	3.83	5.5	438	1.6	33	20	35
LL5	12	0.54	0.30	0.00	8.54	6.4	427	2.5	37	15	37
LL4	12	0.66	0.30	0.00	3.24	5.6	375	2.5	37	15	30
LL3a	12	0.46	0.24	0.00	7.68	5.2	337	2.4	42	18	31
Ka1	9	0.34	0.18	0.00	5.76	3.9	253	1.8	34	19	29

TABLE 3. Nutrient (mmol m^{-3}) and chlorophyll a (mg m^{-3}) concentrations and dissolved inorganic nitrogen (DIN = NO_2 + NO_3 + NH_4) to phosphorus (DIP = PO_4) ratio below the thermocline, average of 20 m and 30 m.

Transect II (4.-5.8.1990)

Station	PO_4	NO_2	NO_3	NH_4	totP	totN	chl *a*	DIN/DIP
LL13	>1.13	0.01	1.10	0.02	1.70	24	0.13	1
LL12	0.23	0.14	0.80	0.00	0.43	27	0.66	4
LL11	0.31	0.16	2.91	0.99	0.56	27	-	13
LL10	0.18	0.08	1.07	0.65	0.44	25	0.67	10
LL9	0.13	0.06	0.33	0.87	0.46	28	1.04	10
LL8	0.39	0.10	4.43	0.00	0.67	30	-	12
LL7	0.39	0.15	4.55	0.12	0.59	27	0.40	12
LL6	0.51	0.11	6.57	0.25	0.77	31	0.43	13
LL5	0.33	0.09	5.30	0.32	0.45	27	0.51	17
LL4	0.48	0.06	7.58	0.26	0.56	32	0.52	16
LL3a	0.36	0.06	5.20	0.15	0.49	31	0.46	15
Kal	0.64	0.07	7.49	0.35	0.88	30	0.91	12

Transect VI (10.-12.8.1990)

Station	PO_4	NO_2	NO_3	NH_4	totP	totN	chl *a*	DIN/DIP
LL13	0.30	0.19	2.24	0.51	0.50	24	1.32	10
LL12	0.49	0.18	3.20	0.86	0.58	30	0.46	9
LL11	0.21	0.08	1.66	0.66	0.45	27	0.41	11
LL10	0.28	0.10	2.38	0.37	0.37	28	2.04	10
LL9	0.20	0.10	2.08	0.37	0.31	24	2.54	13
LL8	0.49	0.05	6.72	0.04	0.54	27	0.61	14
LL7	0.33	0.07	5.52	0.03	0.43	27	0.84	17
LL6	0.40	0.07	4.00	0.33	0.51	42	0.70	11
LL5	0.22	0.09	2.66	0.30	0.40	36	0.64	14
LL4	0.32	0.07	5.87	0.47	0.46	36	0.34	20
LL3a	0.36	0.06	5.20	0.15	0.49	31	0.36	15
Kal	0.41	0.06	5.20	0.14	0.49	31	0.90	13

4.2.3. Chlorophyll, particles and phytoplankton. The division of the Gulf of Finland into sub-basins is clearly reflected in the upper mixed layer chlorophyll *a* and particle concentrations that were determined continuously from the flow-through water. The chlorophyll *a* concentrations ranged from about 4 mg m^{-3} at the westernmost frontal area to 2.5 mg m^{-3} in the easternmost basin (Fig. 4b). The correlation between salinity and chlorophyll was positive but between chlorophyll and temperature negative (Fig. 5).

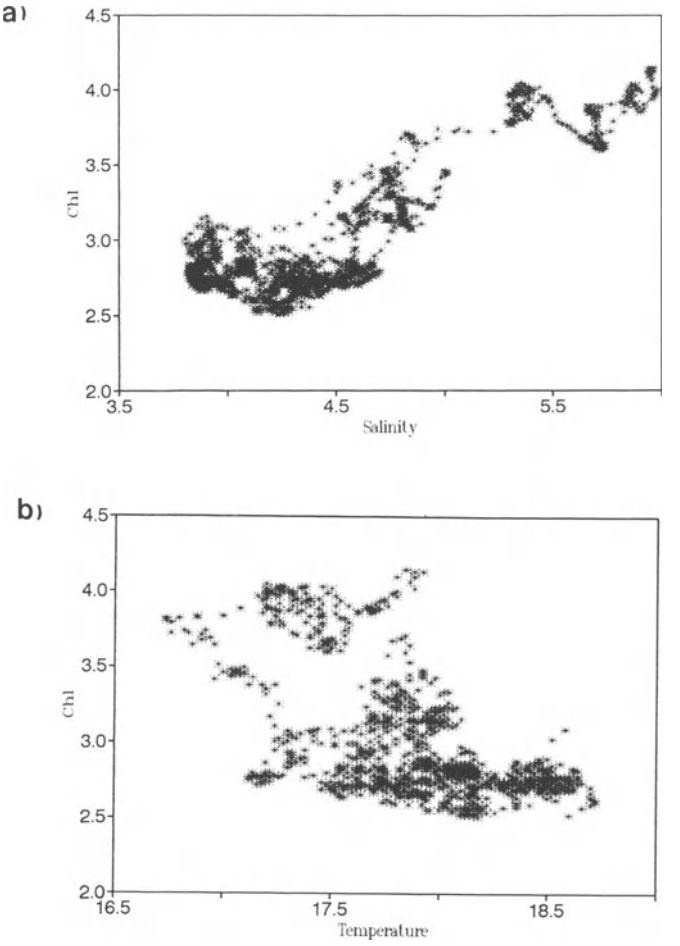

Figure 5. Plots of a) salinity versus chlorophyll and b) temperature versus chlorophyll from the data obtained from the continuous measurements during transect II (4.-5.8.1990). Regression equations: a) chl *a* = 0.08 + 0.65 × sal, r^2 = 0.74, n = 997 b) chl *a* = 13.4 - 0.61 × temp, r2 = 0.34, n = 997.

The particle size class typical of filamentous cyanobacteria (spherical size diameter 10-30 μm) had a definite maxima in the frontal interfaces (Fig. 6b), whereas the smallest size class (2-6 μm, mainly representing auto- and heterotrophic nanoflagellates) well reflected the distribution of water masses in the different sub-basins (Fig. 6a).

At the beginning of the study period all the dominating phytoplankton species showed unique, front-related distribution patterns. *Aphanizomenon flos-aquae*, which was the most abundant species in the samples, was observed in highest density at the two frontal stations: 2 to 4 times higher than at the neighbouring stations (Fig. 7a). The same distribution pattern prevailed throughout the whole study period. However, in the westernmost station, separated from the Gulf of Finland by a front, the abundance increased three-fold after the wind mixing episode during August 4-5. The comparatively low abundance of *Nodularia spumigena* and its sporadic distribution pattern made it difficult to relate the results to water structure (Fig. 7b). The mixing event that occurred between transect II and

IV resulted in low numbers of *Nodularia* filaments at 5 m depth at all stations. This may be due to the mixing of surface aggregates into the whole mixed layer, this species is known to be concentrated in the surface (Niemistö et al. 1989). At the beginning of the study period *Gomphosphaeria lacustris* showed only one maximum inside the low salinity water filament of coastal origin (station LL8). However, the maximum disappeared before the end of the period (Fig. 7c).

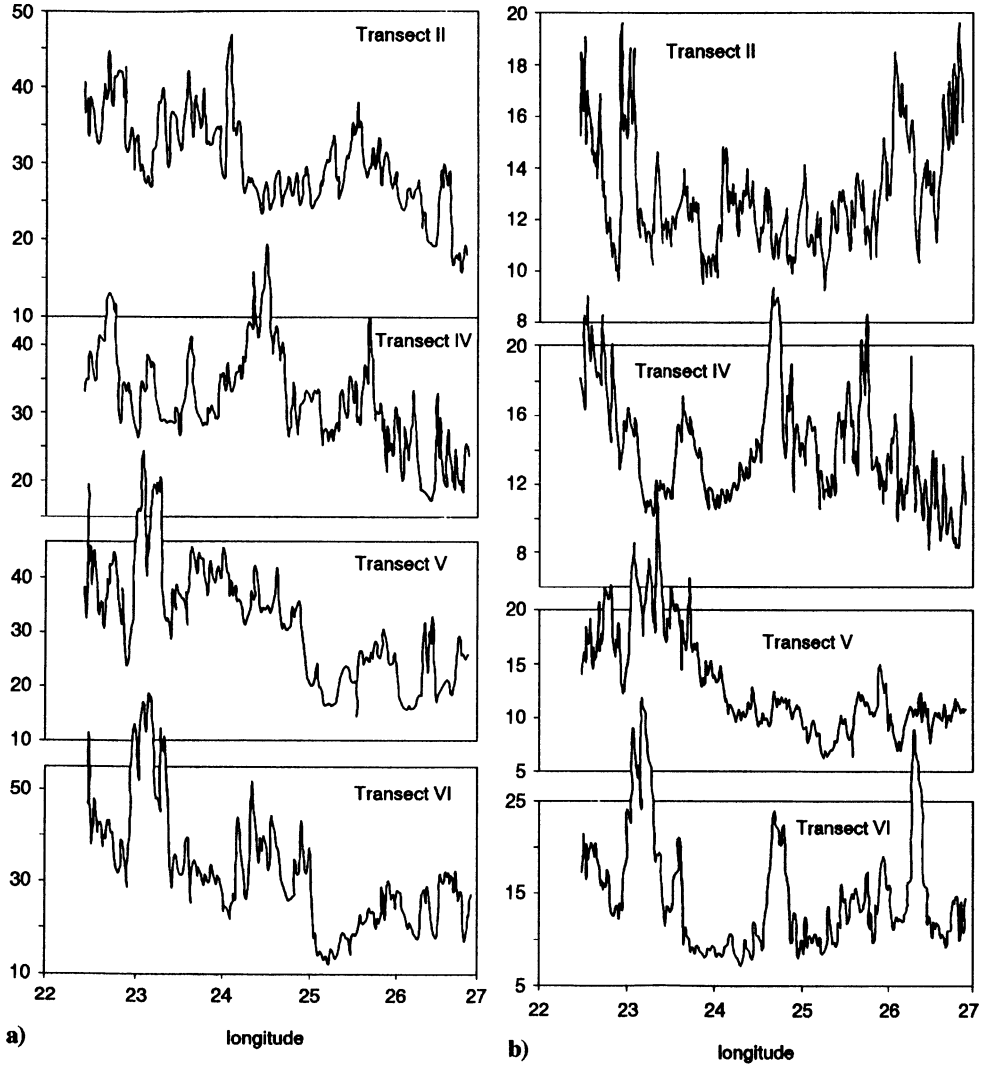

Figure 6. Distribution particles in the size classes a) 2-6 μm and b) 10-30 μm (10^3 dm^{-3}) along the central axis of the Gulf of Finland during 10-12.8.1990.

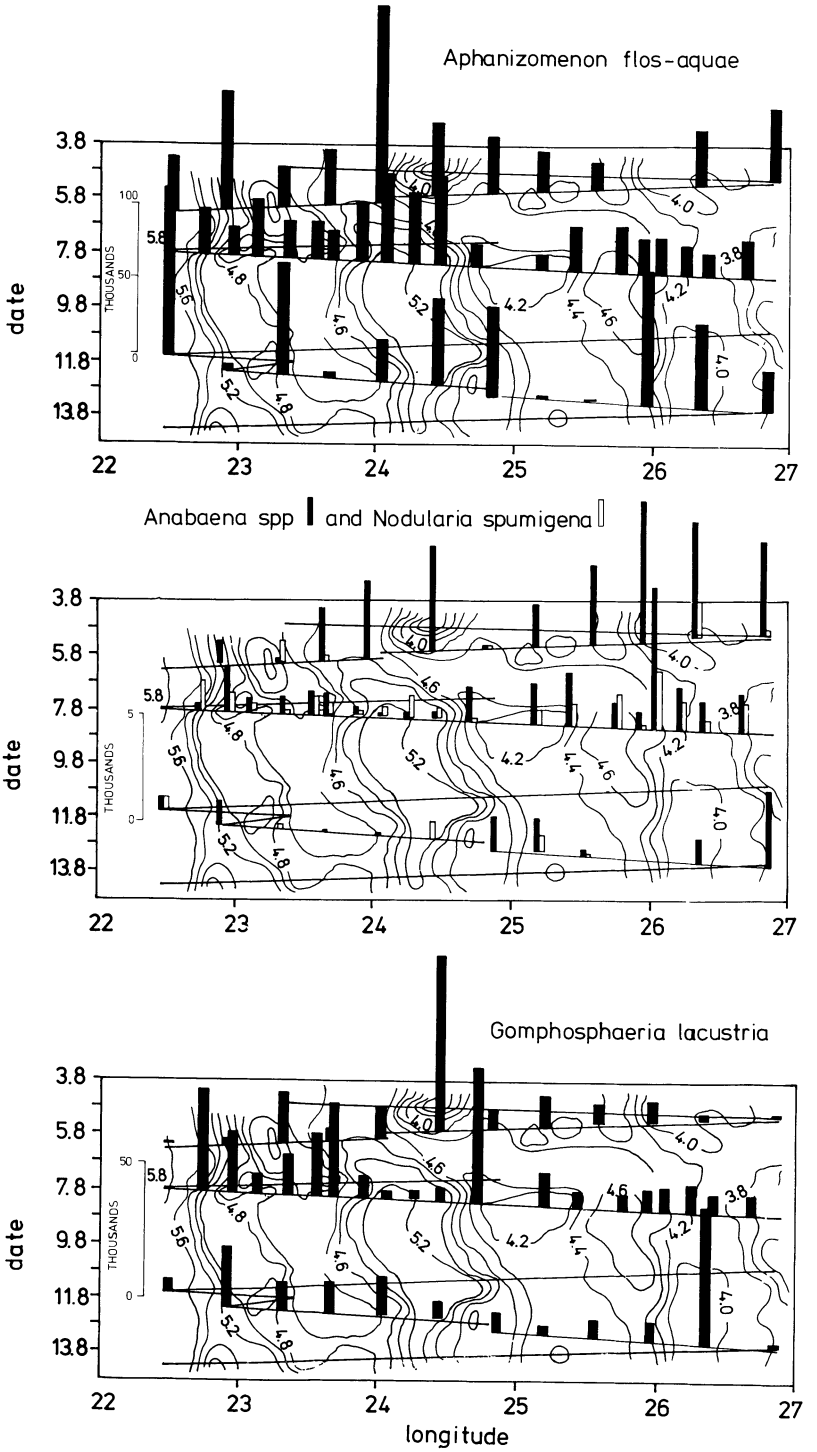

Figure 7. Spatio-temporal distribution of the dominating phytoplankton species along the central axis of the Gulf of Finland (number of 100 μm filaments or colonies per dm^3).

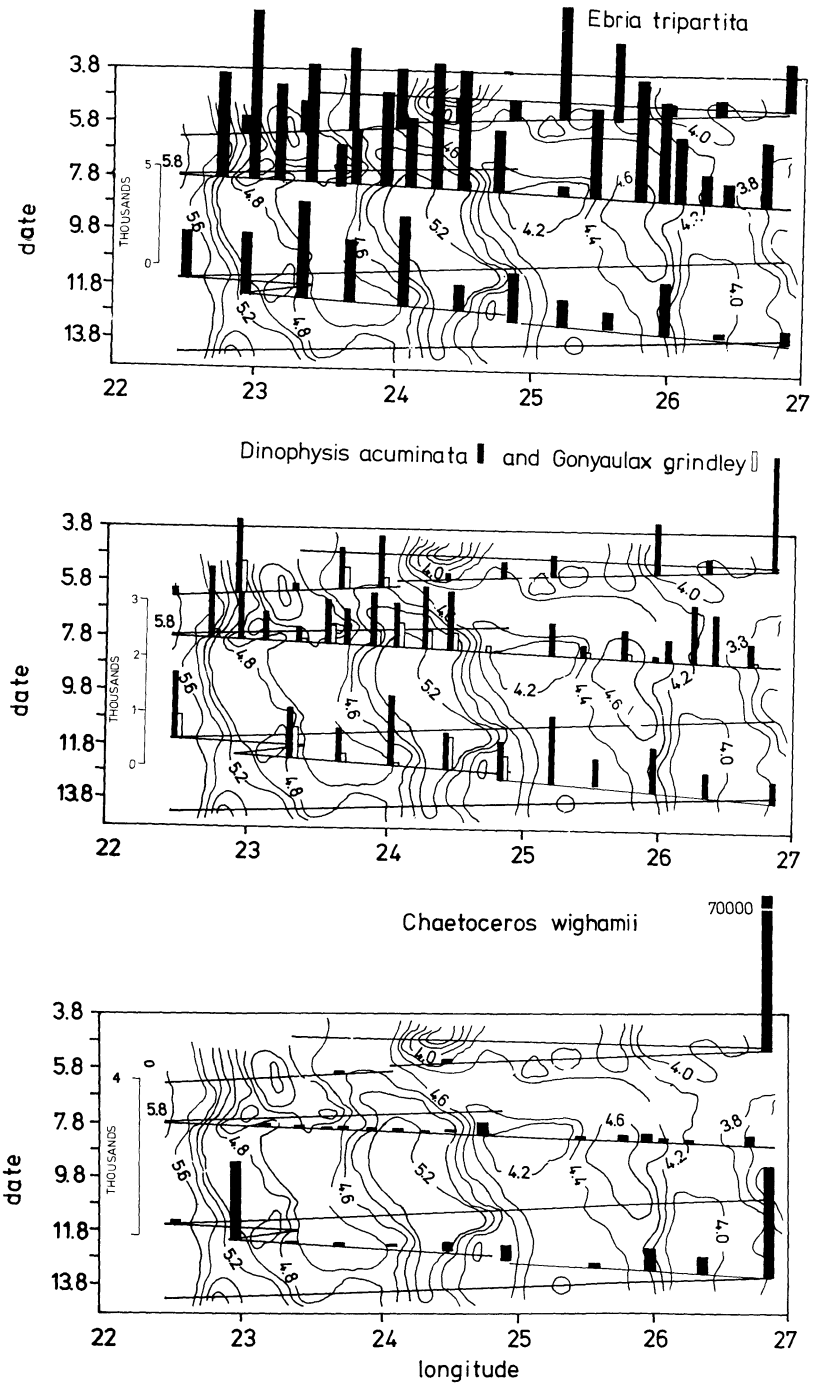

Figure 7. (Continued). Spatio-temporal distribution of the dominating phytoplankton species along the central axis of the Gulf of Finland (cells per dm^3).

The dinoflagellate *Dinophysis acuminata* had the highest cell numbers within the two main frontal areas, as well as within the weaker eastern front, during all the investigated transects (Fig. 7e). At the coastal station, Ka1, a maximum was found only during the beginning of the study period. *Dinophysis norwegica* was found only in the western high salinity water (station LL13). The eastern side of the central frontal system formed a border to the distribution of *Gonyaulax gridleyi* during all the transects. At the beginning of the period the phagotrophic dinoflagellate, *Ebria tripartita*, was found in highest abundances at the stations located within the watermasses between the fronts (Fig. 7d). The wind mixing clearly increased its abundance at all stations, both within the frontal and interfrontal areas.

The diatom *Chaetoceros wighamii* was only found at the eastern coastal station (Ka1), being heavily covered by flagellates (Fig. 7f).

5. Discussion

The close coupling of physical and biological processes is a well recognized but less studied fact in the Baltic Sea. The shallowness, complicated bottom topography and salinity gradients promote the complex dynamics of different water masses that is reflected in the considerable patchiness of plankton well evidenced e.g. in satellite images (Horstmann 1983). An overview of the physical control of the phytoplankton growth in the sea area has been given by Nõmmann (1990).

High primary production has been reported in frontal zones in several deep and shallow seas (e.g. Iverson et al. 1979, Parsons et al. 1981, 1983, Denman and Powell 1984, Holligan et al. 1984, Hesse et al. 1989). Patchiness in both chlorophyll and phytoplankton species distribution have been found to be causally associated with front-related upwellings (Seliger et al. 1981). Studies on the utilization of different nutrient pools in other estuarine basins have shown clear differences in the nutritional dynamics of the pelagic community in frontal and stratified zones (Price et al. 1985, Cochlan et al. 1991).

The association of cyanobacterial blooms with upwelling centres was suggested already in the 1970s (Niemi 1976, Jansson 1978). Kahru et al. (1984) studied the cyanobacterial bloom distribution across a front in the southern Baltic Proper and found 7-fold productivity associated with the frontal upwelling. In the opening to the Gulf of Finland, Kahru et al. (1986) observed a sharp salinity front which was associated with a fluorescence maximum and marked the boundary between different successional stages of a vernal bloom. Our results complement these findings and indicate that fronts play a crucial role in cyanobacterial bloom formation also in the Gulf of Finland.

The time period for the basin-wide scale measurements was 12 days, during which two transects with a complete set of measurements, including vertical profiles of hydrography and nutrients, were made. This relatively short period does not alone allow conclusions to be drawn about the biological and nutrient dynamics over time in the whole basin. In the eastern interfrontal basin, at station LL6, however, a longer 37-day study was carried out. The results showed that our study period was preceded by a strong wind event two weeks earlier, leading to considerable erosion of the seasonal thermocline (from 10 to 20 m at the station LL6) and considerable enrichment of the euphotic layer with inorganic nutrients (Kononen et al, in prep.). A marked increase in cyanobacteria, mainly *Aphanizomenon flos-aquae*, was observed very soon after the mixing event. The positive heat flux through the surface caused the re-establishment of the seasonal thermocline down to depths of 9-13 m prior to our study period. The cyanobacterial population in the interfrontal basin was in the declining phase at the beginning of the basin-wide transects described in this paper.

During the relatively stationary period at the beginning of our surveys the front-related differentiation of water masses regulated the cyanobacterial bloom dynamics in the different subareas. The westernmost and the central quasi-stationary fronts were characterized by high chlorophyll *a*, maxima in total and particulate nutrients, a minimum in ammonium, clear patches in particles of the filamentous cyanobacteria size class, coinciding with maxima of *Aphanizomenon flos-aquae*. Of the other investigated phytoplankton species, *Dinohysis acuminata* had a maximum but the heterotophic *Ebria tripartita* a minimum in the frontal areas. Relative minima in bacterial production and maxima in the autotrophic/heterotrophic flagellate ratio were observed during transect II (August 4-5) within stations situated in the frontal areas, and the opposite in interfrontal stations (Dr. Jorma Kuparinen, Finnish Institute of Marine Research and Dr. Peter Björnsen, University of Copenhagen, unpublished data).

The cyanobacteria represent a special type of new production, sustained by nitrogen fixation. Our results indicate that, even during relatively stationary weather conditions, the proportion of this new production to production based on nutrient regeneration has relatively higher importance in frontal areas than interfrontal basins. This new production is maintained by frontal upwelling. Within the interfrontal basins, incontrast, nutrient pulses due to wind-induced mixing seem to play the major role in bloom initiation. The significance of the two main types of external energy input controlling phytoplankton growth sensu Margalef (1978) (turbulence generated and spread from the surface and upwelling) are thus spatially differentiated during the cyanobacterial bloom in the Gulf of Finland.

The eastern parts of the Gulf of Finland are the most eutrophicated areas due to the heavy nutrient load (Nehring et al. 1990). The most intense and noxious blooms usually develop in the eastern end of the Gulf (e.g. Pitkänen et al. 1990). Our measurements, however, had relatively good correlations between upper layer chlorophyll and salinity and temperature. This can partly be explained by the vertical mixing processes (especially in frontal areas) that lead to more saline and cooler waters and provide nutrient inputs for phytoplankton growth. It can be assumed that the bloom maximum was not reached during the course of our study period.

One striking feature was the differentiation of the subbasins and fronts according to the inorganic nutrient ratio in the water layers below the seasonal thermocline. Adjacent to the westernmost front, the phosphate values below the thermocline (20 m) were as high as those measured a few days before transect I at a depth of 80 m in the Gotland Deep (unpublished data of the Finnish Institute of Marine Research). This suggests that vertical transportation of Baltic Deep water had taken place before the start of our study. The westernmost frontal system, however, seemed to be a border for this transport. The occurrence of the heavy blooms in the Baltic Proper and western Gulf of Finland has traditionally been explained by the intrusions of the phosphorus-rich Baltic deep water (e.g. Niemi 1976, 1982, Niemistö et al. 1989). According to Matthäus (1990), the mechanism for mixing across the primary halocline has been assumed to be internal wave breaking, caused by vertical current shear or internal waves striking the slope. It can be expected that if the stagnation period of the water inflow from the North Sea ends in the future, the followed increase in the salinity of the Baltic Sea will be accompanied by penetration of the halocline inner to the Gulf of Finland, as was in early 1970s. It can be assumed that this leads to most intense blooms in the western and central areas. The dynamics of the vertical exchange processes at the entrance to the Gulf of Finland seem to hold a key position in understanding the cyanobacterial blooms throughout the whole Gulf of Finland and should be studied in more detail.

The eastern basin was characterized by higher concentrations of inorganic nitrogen, mostly nitrate, and a high N:P ratio below the thermocline. The origin of this large nitrogen reserve is unclear. However, we did not measure nutrients down the whole water body, but only the upper 30 m layer. It can, however, be assumed that the high nitrogen values are connected with the nutrient load. Long-term monitoring data from the Gulf of Finland show that the total nitrogen concentrations have

doubled since the middle of the 1970's, whereas there are no clear trends in phosphorus levels (Baltic Sea Environment Protection Commission 1990, unpublished data of the Finnish Institute of Marine Research) and hence the N:P ratio has increased.

An often cited hypothesis about the initiation of the cyanobacterial bloom in the Baltic Sea is the one presented by Schindler (1977) for fresh waters and Niemi (1979) for the Baltic Sea. According to this hypothesis, the low N:P ratio, characteristic for the Baltic Sea surface waters, results in nitrogen limitation of phytoplankton growth and is therefore associated with the dominance of nitrogen-fixing cyanobacteria. The data presented by Niemi (1979) showed that the occurrence of the bloom throughout the whole Baltic Sea area is areally consistent with the N:P ratio. An interesting debate has recently arisen about whether the hypothesis is valid in estuaries like the Baltic Sea (Smith 1990, Howarth and Marino 1990). In the Gulf of Finland, according to our results and the unpublished nutrient results of the Finnish Institute of Marine Research, the highest N:P ratios are found in the eastern areas, where the most noxious blooms usually develop. According to Pitkänen et al. (1990), both heterosystous and nonheterosystous species are present in the blooms in that area. This is in contradiction with the above-mentioned hypothesis. The pelagial biology of the eastern Gulf of Finland is poorly studied. Nutrient enrichment experiments involving the simulation of nutrient pulses below the thermocline water have not been carried out in this area, only at the entrance to the Gulf of Finland (Tamminen et al. 1985, Pelag 1990) and in the archipelago off Helsinki (Rinne and Tarkiainen 1978). It would appear that the controlling role of nutrients is a more complicated question than previously thought. More definite studies are needed about the bloom dynamics especially in the eastern Gulf of Finland.

6. Conclusions

Much of the variability in the pelagial ecosystem of the Gulf of Finland during summer can be attributed to the separation of the area into sub-basins by at least three quasi-stationary frontal systems. The late summer cyanobacterial bloom in the area is controlled by the external energy inputs sensu Margalef (1978). In the stratified interfrontal basins, the wind-induced turbulence plays the major role in nutrient flux from below the seasonal thermocline to the euphotic layer. The frontal upwelling maintains high cyanobacterial production during calm periods. The nutrient ratios below the seasonal thermocline differ between the inter-frontal basins and fronts, and thus nutrient pluses from water below the seasonal thermocline caused by external energy inputs are expected to have unequal biological consequences in different subareas. The information about the physical and hydrographic structure of subareas is essential for the large-scale modelling of the Baltic Sea ecosystem.

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N_2 FIXATION IN NON-HETEROCYSTOUS CYANOBACTERIA: AN OVERVIEW

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ABSTRACT. Studies on N_2-fixing non-heterocystous cyanobacteria have centred on strains that can fix N_2 aerobically. Extensive laboratory studies, reviewed here, have probed the mechanisms whereby these organisms maintain an active nitrogenase in the presence of both atmospheric and photosynthetic O_2, the intracellular location of nitrogenase and the supply of ATP, reductant and carbon skeletons to support N_2 fixation. In contrast, field studies on natural populations of non-heterocystous cyanobacteria have been less widespread, though these organisms make a major contribution to N_2 fixation in the marine environment. Most fieldwork has concentrated on microbial mat communities and on open-water blooms of *Trichodesmium*, in which both the extent and the pattern of observed N_2 fixation are markedly influenced by external factors such as light, O_2 and CO_2. The differences in behaviour between *Trichodesmium* and other non-heterocystous cyanobacteria are highlighted in this review and possible directions for future research are indicated.

1. Introduction

The ability to fix N_2 aerobically is apparently universal among heterocystous cyanobacteria. In contrast, in their comparative survey of cyanobacterial strains in the culture collection of the Pasteur Institute, Rippka *et al.* (1979) found that only 5 out of 133 strains of non-heterocystous cyanobacteria were capable of aerobic N_2 fixation though rather more strains (51 out of 133) could fix N_2 when grown anaerobically or microaerobically . Since 1979, several more diazotrophic non-heterocystous cyanobacteria have been isolated (see Waterbury *et al.*, 1988; Gallon, 1989; Kostyaev, 1990), but aerobic N_2 fixation remains unusual among these organisms. In our own experience, attempts to isolate aerobic N_2-fixing cyanobacteria from natural populations almost always result in the isolation of heterocystous strains.

Most attention to date has been given to those non-heterocystous cyanobacteria capable of aerobic N_2 fixation; not least because they are unique among diazotrophs in sustaining the O_2-sensitive process of N_2 fixation in the presence of

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both atmospheric and photosynthetic O_2. On the other hand, there have been some laboratory studies on non-heterocystous cyanobacteria such as *Plectonema, Phormidium* and *Pseudanabaena* that, in pure culture, fix N_2 only in the presence of less than 0.2 atm of O_2 (for references see Gallon, 1989; Smoker & Barnum, 1990). Natural populations of these, and similar, microaerophilic cyanobacteria fix N_2 in environments where the prevailing concentration of O_2 is low. Sulphur springs, stagnant ponds and waterlogged soils afford examples of such environments but, in a marine context, microaerobic environments include laminated microbial mats, in which N_2 fixation by microaerophilic cyanobacteria can be substantial (Stal & Krumbein, 1985a; Bebout *et al.*, 1987; Skyring *et al.*, 1988; Paling *et al.*, 1989), and surface-associated, organic-rich, O_2-depleted microzones (Paerl & Carlton, 1988).

Though aerobic N_2 fixation has been demonstrated in a number of non-heterocystous cyanobacteria, laboratory studies have centred on the unicellular genera *Gloeothece* and *Synechococcus* and on the filamentous genera *Oscillatoria* and *Microcoleus*. *Gloeothece* is not a marine cyanobacterium, though some strains of *Synechococcus* are of marine origin (León *et al.*, 1986; Spiller & Shanmugam, 1987). However, all the *Oscillatoria* strains that have, so far, been studied in the laboratory are marine, having been isolated originally from a cyanobacterial mat located on a sandy island in the North Sea (Stal & Krumbein, 1981; Stal *et al.*, 1984), a temperate salt marsh (Griffiths *et al.*, 1987) or from a tropical mangrove swamp (Gallon *et al.*, 1991b). Similarly, *Microcoleus chthonoplastes* was originally isolated from an intertidal mudflat (Malin & Pearson, 1988). It should, nevertheless, be noted that all of these marine strains, unicellular or filamentous, originated from either the littoral fringes of the marine environment, or were found attached to marine plants or other surfaces. The only well-documented example of a marine non-heterocystous cyanobacterium that fixes N_2 in the open ocean is *Trichodesmium* and, though it has been grown in the laboratory (Ohki *et al.* 1986), most studies with this cyanobacterium have used natural populations (Dugdale *et al.*, 1961; Taylor *et al.*, 1973; Carpenter & Price, 1976; Bryceson & Fay, 1981; Carpenter *et al.*, 1990; Capone *et al.*, 1990).

2. Laboratory studies

Over the years, non-heterocystous N_2-fixing cyanobacteria have attracted a number of questions. Among the most persistent of these questions have been the following:

'Given the well-documented O_2-sensitivity of N_2 fixation, how do non-heterocystous cyanobacteria maintain an active nitrogenase whilst photoevolving O_2?'

'Where is nitrogenase located in non-heterocystous cyanobacteria?'

'Does photosynthesis directly supply ATP and reductant for N_2 fixation in

non-heterocystous cyanobacteria?'

In attempting to answer these, and other, questions much has been learned about the behaviour of N_2-fixing non-heterocystous cyanobacteria and in this chapter we aim to present a summary of what has been achieved to date. However, in the space available to us, it is impossible to cover all the laboratory work that has been done on non-heterocystous N_2-fixing cyanobacteria during the last 20 years. Much of this work appears already in review form (Gallon & Chaplin, 1988a, and earlier reviews cited therein; Gallon & Chaplin, 1988b; Gallon, 1989; Gallon, 1990) so, in order to avoid repetition, points covered in more detail in the above works will receive only brief mention here. In addition, since the molecular biology of cyanobacterial N_2 fixation is covered elsewhere in this volume (see chapter by Zehr), this topic will not be dealt with here. Rather, we hope to indicate how the results of laboratory studies on the mechanism of N_2 fixation in non-heterocystous cyanobacteria, though based on non-marine or benthic organisms, might nevertheless help us to understand the process in the rather more intractable planktonic marine cyanobacteria.

2.1 HOW DO NON-HETEROCYSTOUS CYANOBACTERIA MAINTAIN AN ACTIVE NITROGENASE WHILST PHOTOEVOLVING O_2?

Like other diazotrophs, non-heterocystous cyanobacteria employ a variety of strategies in order to limit the deleterious effects of atmospheric O_2 on N_2 fixation (Gallon, 1989). These strategies include avoidance (noteworthy among microaerophiles), O_2-consuming reactions that maintain a low intracellular concentration of O_2, removal of toxic O_2-derived radicals, 'conformational' protection, as in *Azotobacter* (see, for example, Hill, 1988), and continuous synthesis of nitrogenase in order to replace O_2-inactivated enzyme. The special role of boron in the protection of nitrogenase from inactivation by O_2 in heterocystous cyanobacteria does not extend to non-heterocystous strains (Bonilla *et al.*, 1990). Not all of these strategies are found in every non-heterocystous cyanobacterium and, moreover, subtile differences in detail exist. In *Gloeothece* for example, the dominant strategies appear to be carbon-supported O_2-consumption and *de novo* synthesis of nitrogenase, whilst in *Oscillatoria limosa* (previously *Oscillatoria* strain 23) these are augmented by an O_2-induced 'conformational' change in nitrogenase that allows immediate recovery of activity once O_2-stress is removed (Stal & Krumbein, 1985b). However, in *Synechococcus* 43511 (Reddy & Mitsui, 1984) and in *Trichodesmium* (Saino & Hattori, 1982; Carpenter *et al.*, 1990) H_2-linked O_2-consumption appears to be important in the maintenance of aerobic N_2 fixation.

In at least two species of the heterocystous cyanobacterium, *Anabaena*, the presence of superatmospheric concentrations of O_2 results in an apparently reversible modification of the Fe-protein of nitrogenase that alters its electrophoretic mobility when subjected to SDS-PAGE. The extent to which this modification correlates with nitrogenase activity is not, however, clear. In *Anabaena variabilis*, the modified (slower migrating) form is present at only low concentrations in aerobically grown cultures and may be catalytically inactive (Reich & Böger, 1989; Ernst *et al.*, 1990).

On the other hand, in *Anabaena* CA, the modified form predominates in cultures grown in air and also in cultures exposed to O_2:CO_2 (99:1) that nevertheless actively fix N_2. In this cyanobacterium, the modified Fe-protein may retain its catalytic activity in addition to becoming O_2-stable (Smith *et al.*, 1987), though this has not actually been demonstrated. The presence of two forms of the Fe-protein of nitrogenase in *Oscillatoria limosa* (Stal & Bergman, 1990) extends these observations to non-heterocystous cyanobacteria and may be of particular relevance to the earlier work in *O. limosa* implying 'conformational' protection (Stal & Krumbein, 1985b). As in *Anabaena* CA, most, though not all, of the Fe-protein in aerobic cultures of *Oscillatoria limosa* was in the slower migrating, modified form (Stal & Bergman, 1990). Because nitrogenase activity was markedly stimulated by transfer to anaerobic conditions (Stal & Krumbein, 1985b), the modified form of the Fe-protein of nitrogenase may be catalytically inactive in *O. limosa*, as in *A. variabilis*. Modification of the Fe-protein of nitrogenase may also occur in *Trichodesmium* (Capone *et al.*, 1990; Zehr, this volume).

In contrast to its effect in other diazotrophs, O_2 does not permanently repress nitrogenase synthesis in *Gloeothece* (Maryan *et al.*, 1986). The transient effect of O_2 on nitrogenase synthesis differs from that of NH_4^+, which represses synthesis permanently, suggesting that the mechanisms of repression differ. Studies on DNA topoisomerases have provided circumstantial evidence that the transient repression of nitrogenase synthesis in *Gloeothece* by O_2 is effected through a transient relaxation of one or more of the *nif* genes (Gallon *et al.*, 1989; Gallon, 1990).

Previous work suggesting that respiratory consumption of O_2 may be important in the maintenance of aerobic N_2 fixation in *Gloeothece* (see Gallon, 1989) has been extended by the observation that the higher rate of respiration seen in N_2-fixing cells (relative to that in cells grown on NO_3^-) was due largely to a stimulation of uncoupled respiration located in the non-photosynthetic plasma membrane (Peschek *et al.*, 1991). This respiration may serve the dual role of consuming O_2 and fuelling transport processes across the plasmalemma. In this context it may be significant that N_2-fixing cultures of *Gloeothece* release and reassimilate amino acids (Flynn & Gallon, 1990). It should, however, be noted that O_2 consumption by diazotrophs is not confined to respiratory electron transport. Under appropriate conditions, nitrogenase itself may catalyze an O_2-consuming reaction (Thorneley & Ashby, 1989).

Non-heterocystous cyanobacteria must cope not only with atmospheric O_2 but also with their own photoevolved O_2. When exposed to alternating light and darkness, most laboratory cultures fixed virtually all of their N_2 during the period of darkness (Gallon, 1989), thereby effecting a temporal separation between N_2 fixation and photosynthetic O_2 evolution. However, this behaviour was not found in all non-heterocystous cyanobacteria. For example, in laboratory cultures of *Trichodesmium*, N_2 fixation is light-dependent (Ohki & Fujita, 1988) and, in natural populations, confined to the daytime (Bryceson & Fay, 1981; Saino & Hattori, 1978; Capone *et al.*, 1990). In addition, Jones (1990) demonstrated light-dependent N_2 fixation in natural populations of a strain of *Lyngbya majuscula* that, unusually for this genus

(Rippka *et al.*, 1979), was capable of aerobic N_2 fixation. It is not clear why these cyanobacteria should differ from other non-heterocystous strains, though it may be significant that *Trichodesmium* and the *Lyngbya* strain both occur in wave-aerated seawater.

The usual pattern of N_2 fixation found in *Gloeothece* incubated under alternating light and darkness (N_2 fixation during the period of darkness) can be altered in response to changes in environmental conditions. For example, in marked contrast to aerobically grown batch cultures and natural populations of *Gloeothece*, continuous cultures fixed N_2 predominantly during the period of illumination (Ortega-Calvo & Stal, 1991). Batch cultures of *Gloeothece* incubated microaerobically also fixed N_2 during the light period (Gallon, 1989). It should also be pointed out that cultures of cyanobacteria that fix N_2 only during the dark phase of a cycle of alternating light and darkness nevertheless simultaneously fix N_2 and photoevolve O_2 when incubated in the laboratory under continuous illumination. The temporal separation observed under alternating light and darkness is not therefore obligatory for aerobic N_2 fixation, though there is no doubt that it is widespread among natural populations of non-heterocystous cyanobacteria.

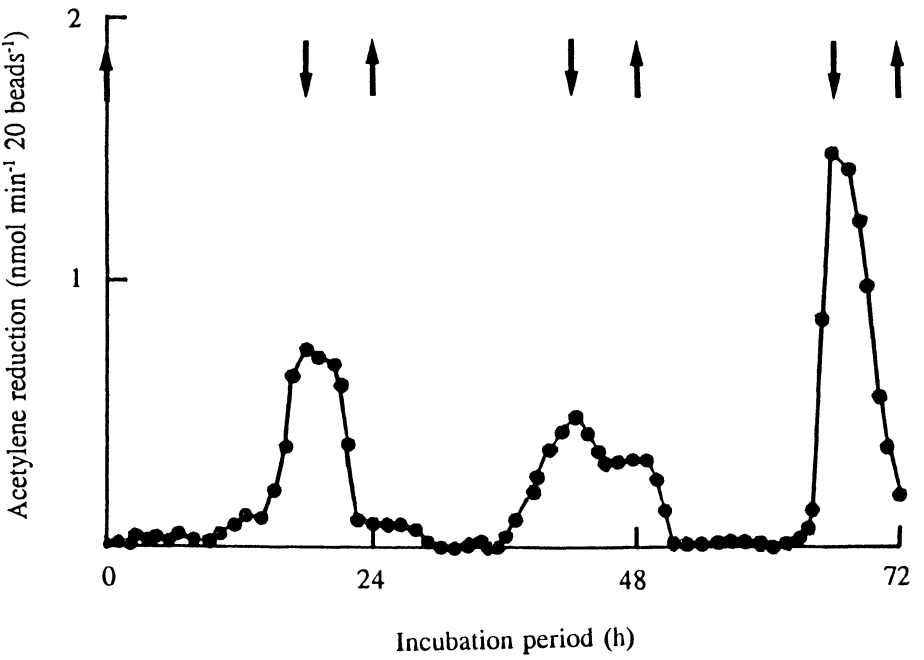

Fig. 1. Effect of 2 mM NH_4Cl on N_2 fixation (acetylene reduction) by cultures of *Gloeothece* immobilized in beads of calcium alginate (Gallon *et al*, 1988) and incubated under continuous illumination of 25 μmol photons m^{-2} s^{-1}. NH_4Cl was added where indicated (|); 6h later cultures were transferred to fresh medium (|). Data courtesy of Mr. J.S. Yunes.

2.2 DOES PHOTOSYNTHESIS DIRECTLY PROVIDE ATP AND REDUCTANT FOR N_2 FIXATION?

In theory, the location of nitrogenase within the same cell as the photosynthetic machinery would allow the photolysis of water to provide directly the reducing power for N_2 fixation. In addition, photophosphorylation could meet the ATP requirement of nitrogenase. However, in those non-heterocystous cyanobacteria that fix N_2 only during the dark phase of a cycle of alternating light and darkness, it is clear that photosynthesis does not always directly support N_2 fixation. For example, in *Gloeothece* incubated under 12h light : 12h darkness, photosynthesis functions to synthesize storage glucan during the light phase, with breakdown of this glucan supporting N_2 fixation during the period of darkness (Gallon *et al.* 1988). However, *Gloeothece* cannot grow at the expense of exogenously supplied carbon sources. On the other hand, cultures of *Oscillatoria* spp. UCSB8 and UCSB25 could grow and fix N_2 both photoheterotrophically (in the light in the presence of DCMU to inhibit photosynthesis) and heterotrophically (in the dark) when supplemented with suitable sugars (Gallon *et al.* 1991b). Exogenous sugars were catabolized through the operation of the pentose phosphate pathway, which could provide both the reductant and the ATP needed for N_2 fixation. Externally supplied sugars may also support microaerobic N_2 fixation in cultures of *Plectonema boryanum* (Smoker & Barnum, 1990). However, N_2 fixation under truly anaerobic conditions (in the absence of both atmospheric and photosynthetic O_2) appears to be unusual among non-heterocystous cyanobacteria, though *Oscillatoria limosa* can fix N_2 anaerobically in the dark (Stal & Heyer, 1987). Under these conditions, *O. limosa* ferments intracellular trehalose (which functions as an osmoregulant in this cyanobacterium) to acetate (Heyer *et al.* 1989).

It is clear therefore that many non-heterocystous cyanobacteria can support N_2 fixation by catabolism of either storage reserves or, in some cases, at the expense of exogenously supplied carbon compounds. The question arises, however, as to whether photosynthesis directly supports N_2 in cyanobacteria like *Trichodesmium*, that fix N_2 during the light phase of an alternating cycle of light and darkness and, indeed, in other non-heterocystous cyanobacteria when incubated under continuous illumination. This is a difficult question to answer, because even when photosynthesis is occurring simultaneously with N_2 fixation, the link between the two processes may be indirect, *via* fixed carbon compounds. Thus, stimulation of N_2 fixation by light may be due to increased availability of fixed carbon or even to increased availability of O_2 to support aerobic carbon catabolism rather than to direct photosynthetic provision of ATP and reductant for nitrogenase. A variety of laboratory studies with *Gloeothece* (see Gallon, 1989) gave the impression that photosynthesis did not directly supply either reductant or ATP for N_2 fixation. On the other hand, this may depend on precise physiological and environmental conditions (Gallon, 1989; Ortega-Calvo & Stal, 1991).

In non-heterocystous cyanobacteria that, under alternating light and darkness, fix N_2 in the dark, the mechanism underlying the process varies from organism to organism, though it always seems to involve regulation of nitrogenase synthesis (for details and primary refences, see Gallon, 1989). In *Synechococcus* spp. and in *Oscillatoria limosa* (Stal & Krumbein, 1985c), the pattern of N_2 fixation imposed under alternating 12h light and 12h darkness persisted when cultures were transferred to constant illumination. Furthermore, in one strain of *Synechococcus*, this induced diurnal rhythm has been explained in terms of synchronous cell division, with N_2 fixation confined to a specific phase within the cell cycle (Mitsui *et al.*, 1986). On the other hand, in both *Gloeothece* and *Oscillatoria* sp UCSB8, the pattern of N_2 fixation imposed by alternating light and darkness did not persist following transfer to constant illumination (Gallon & Chaplin, 1988a; Gallon *et al.*, 1991b). In *Gloeothece*, the diurnal pattern of N_2 fixation seen under alternating 12h light and 12h darkness appears to be related to the availability of storage glucan, which supports both nitrogenase synthesis and activity in the dark (Gallon *et al.*, 1988). However, in *Oscillatoria* sp. UCSB8, this seems not to be the case and the pattern of N_2 fixation seen under alternating light and darkness may rather reflect alternating periods of nitrogen repleteness and limitation (Gallon *et al.*, 1991a; 1991b). Addition of NH_4^+ for 6h out of each 24h period induced a diurnal rhythm of N_2 fixation in cultures of *Gloeothece* incubated under continuous illumination (Fig. 1), so fluctuations in intracellular nitrogen status can also affect N_2 fixation in this cyanobacterium. However, no fluctuations in the intracellular nitrogen status of *Gloeothece* were observed in cultures growing under alternating light and darkness. Instead, cultures appeared permanently nitrogen-stressed (Flynn & Gallon, 1990).

In *Gloeothece*, and probably in other non-heterocystous cyanobacteria, the rate of protein synthesis is much greater in the light than in the dark. Nitrogen newly fixed during the dark period is not therefore used immediately for bulk protein synthesis, though the immediate product of N_2 fixation (NH_4^+) is rapidly assimilated into glutamine, glutamate and other amino acids. In *Gloeothece*, the intracellular concentrations of amino acids did not fluctuate during growth under alternating light and darkness. On the other hand, marked fluctuations occurred in extracellular glycine, serine and alanine. During the dark period, these amino acids were released into the extensive slime capsule that surrounds *Gloeothece* and, during the period of illumination, they were reassimilated in order to fuel protein synthesis (Flynn & Gallon, 1990). Indeed, about 40% of the protein fixed in the dark appeared as extracellular amino acids. The balance may have been converted into insoluble cyanophycin. Although fluctuations in this reserve material were not observed by Mackerras *et al.* (1990) a slight accumulation of cyanophycin during the period of N_2 fixation might account for at least some of the nitrogen fixed (Gallon *et al.*, 1990). On the other hand, some synthesis of phycobiliproteins (which may act both as a nitrogen reserve and as photosynthetic accessory pigments) may occur during the period of darkness (J.P.H. Reade & J.R. Gallon, unpublished observation). In contrast to the situation in *Gloeothece*, release of amino acids by cultures of *Oscillatoria* sp. UCSB8 was barely detectable and accounted for no more than 0.03% of

nitrogen fixed (O.A. Al-Amoudi, K.J. Flynn & J.R. Gallon, unpublished findings). This difference in behaviour may be a consequence of the different natural environment of these cyanobacteria: nitrogen released by *Gloeothece* growing on rock faces in caves would be available for later reassimilation whilst nitrogen released by *Oscillatoria* growing in a salt marsh environment would rapidly be lost. On this basis, release of newly fixed nitrogen would appear to be an unlikely general strategy for marine cyanobacteria even though, in *Gloeothece*, such behaviour may promote maximum rates of N_2 fixation by maintaining an intracellular environment that is permanently nitrogen-stressed.

Fixation of N_2 by *Trichodesmium* during the hours of daylight is accompanied by synthesis of nitrogenase (Capone *et al.*, 1990). It may therefore be a general property of non-heterocystous cyanobacteria that diurnal fluctuations in N_2 fixation, observed under alternating light and darkness, reflect fluctuations in nitrogenase synthesis, regardless of when N_2 fixation occurs within the illumination cycle. Moreover, fluctuations in nitrogenase activity that are observed in cultures of non-heterocystous cyanobacteria during incubation under continuous illumination (Mullineaux *et al.*, 1981; Stal & Krumbein, 1985c; Huang *et al.*, 1990; Gallon *et al.*, 1991b) may also reflect fluctuations in nitrogenase synthesis. This seems to be the case in *Synechococcus* RF-1 (Huang & Chow, 1990) and, based on incorporation of ^{35}S into the MoFe protein of nitrogenase (Maryan *et al.*, 1986), in *Gloeothece* (Fig. 2). However, unlike synthesis of nitrogenase in *Gloeothece* under alternating light and darkness, fluctuations in nitrogenase synthesis and activity under continuous illumination were not accompanied by fluctuations in storage glucan, respiratory CO_2 or in the intracellular ratio of ADP/ATP (S.M. Perry, A.E. Chaplin & J.R. Gallon, unpublished findings).

2.3 WHERE IS NITROGENASE LOCATED?

The idea that cyanobacteria capable of aerobic N_2 fixation separate nitrogenase from photosynthetic O_2 either in space (for example by confining N_2 fixation to heterocysts) or, in natural populations, in time (fixing N_2 at night) is an attractive hypothesis. However, according to this hypothesis, those non-heterocystous cyanobacteria that fix N_2 during the light period of a cycle of alternating light and darkness ought to effect a spatial separation between N_2 fixation and photosynthesis. This spatial separation could be intercellular, with N_2 fixation confined to cells that, though not differentiated, nevertheless behave like heterocysts and do not produce O_2. Alternatively, the separation could be intracellular, with N_2 fixation located distant from the photosynthetic thylakoid membranes. The early work on *Trichodesmium*, suggesting that N_2 fixation was confined to non-photosynthetic cells in the central region of the bundles (Carpenter & Price, 1976) was consistent with this hypothesis. However, recent studies do not support the view that this central region differs substantially from the rest of the colony, and direct immunolocation of the Fe-protein of nitrogenase in cultures of *Trichodesmium* has shown that, although not all individual trichomes contained nitrogenase, those that did were randomly distributed

within the colony (Bergman & Carpenter, 1991). As far as it was possible to tell, all cells within a nitrogenase-containing trichome possessed the enzyme. Moreover, within individual nitrogenase-containing cells, the Fe-protein appeared homogeneously distributed (Paerl *et al.*, 1989c), suggesting that, unless the distribution of the MoFe-protein of nitrogenase differs from that of the Fe-protein, there is no specific

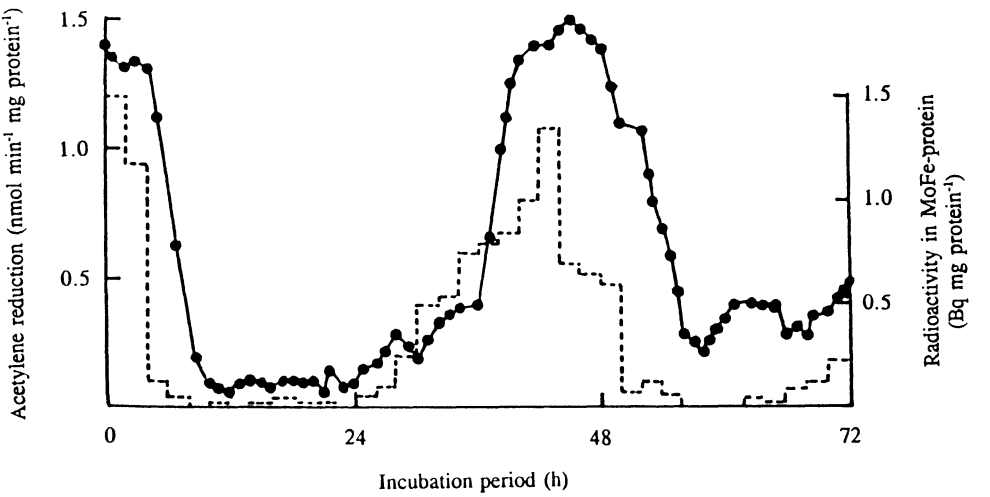

Fig. 2. Activity (•) and synthesis (-----) of nitrogenase in cultures of *Gloeothece* incubated under continuous illumination of 25 μmol photons m^{-2} s^{-1}. Activity was measured as acetylene reduction; synthesis as incorporation of radioactivity from $Na^{35}SO_4$ into the MoFe-protein of nitrogenase (Maryan *et al*, 1986). Data courtesy of Dr. S.M. Perry.

intracellular separation between N_2 fixation and photosynthesis. Nevertheless, because not all cells in a colony of *Trichodesmium* contained nitrogenase, the possibility of intercellular spatial separation of nitrogenase and photosynthetic O_2 evolution remains. It would be interesting to know whether those nitrogenase-containing cells within a bundle of *Trichodesmium* actively photoevolve O_2.

In *Oscillatoria limosa*, the Fe-protein of nitrogenase was found in all cells of the filaments and was evenly distributed within the intracellular material (Stal & Bergman, 1990). Preliminary studies (A. Pettersson, B. Bergman & J.R. Gallon, unpublished) suggest that the same is true of cultures of *Gloeothece*. Similarly, in N_2-fixing cultures of the microaerophilic cyanobacterium *Plectonema boryanum*, nitrogenase was present in all cells (Smoker & Barnum, 1988). However, in all of the above studies it should be noted that the presence of nitrogenase protein within a cell does not guarantee that that cell was actively fixing N_2 or even actively synthesizing nitrogenase.

3. Studies on natural communities

By far most studies on N_2 fixation in natural communities of cyanobacteria have focussed on heterocystous species. However, because many non-heterocystous cyanobacteria are now known to fix N_2, there is a need to elucidate the ecological significance of this group of cyanobacteria. The existence of non-heterocystous cyanobacteria capable of N_2 fixation under fully aerobic conditions further raises the questions why do such organisms not outcompete heterocystous species and why are they present in only a limited number of places.

3.1 IN WHICH ENVIRONMENTS ARE COMMUNITIES OF NON-HETEROCYSTOUS CYANOBACTERIA FOUND?

Among marine plankton of the tropical and subtropical oceans, filamentous *Trichodesmium* spp. are widespread (Carpenter, 1983). *Trichodesmium* forms dense blooms when conditions are calm. The gas-vacuolated trichomes, aggregated in colonies ('tufts') become buoyant and accumulate in the euphotic zone. Blooms of *Trichodesmium* are especially common in oligotrophic waters such as the Sargasso Sea (Carpenter, 1973) and the central North Pacific Ocean (Mague *et al.*, 1977) but they may also occur in more nutrient-rich places. It is generally assumed that the minimum temperature for development of blooms of *Trichodesmium* is 20-25° C and that a salinity of about 3.5% is optimal. It also seems likely that unicellular cyanobacteria contribute to N_2 fixation in the marine plankton. Mitsui and co-workers have isolated a number of N_2-fixing *Synechococcus* strains from several locations in the tropical and subtropical marine environment (Duerr & Mitsui, 1980, Mitsui *et al.*, 1986). However, to our knowledge there are no published reports concerning the environmental significance of natural populations of these organisms.

Marine microbial mats have been shown to include populations of non-heterocystous cyanobacteria. On the shores of Church Island, North Wales, Pearson *et al.* (1979) found nitrogenase activity in microbial mats that were composed mainly of *Microcoleus chthonoplastes*, and showed that this species was capable of aerobic N_2 fixation in culture. Potts *et al.* (1978) concluded that *M. chthonoplastes* was responsible for N_2 fixation in microbial mats from the coast of the German island of Wangeroog, whilst Potts & Whitton (1977) implicated this cyanobacterium in N_2 fixation in microbial mats on Aldabra Atoll. More recently, Skyring *et al.* (1988) reported N_2 fixation in *Microcoleus* mats from Shark Bay, Western Australia. *Microcoleus chthonoplastes* is known as a cosmopolitan cyanobacterium and the most important builder of cyanobacterial mats in the world. However, microbial mats are very often composed of other non-heterocystous cyanobacteria. Stal *et al.* (1984) and Villbrandt *et al.* (1990) found that, on a North Sea island, *Oscillatoria limosa* was the N_2-fixing agent in mats that were predominantly built by *M. chthonoplastes*. In addition, Griffiths *et al.* (1987) reported N_2-fixing populations of *Oscillatoria* sp. from Landimore Marsh in southwest Wales. Other studies are consistent with the

idea that cyanobacteria other than *Microcoleus chthonoplastes* may fix N_2 in microbial mats. For example, Paling *et al.* (1989) reported N_2 fixation in mats from the Dampier Archipelago, Western Australia. Although *in situ* measurements of nitrogenase activity were made only in mats of *M. chthonoplastes*, these authors noted the occurrence in these mats of 5-10% of *Oscillatoria* sp. Gotto *et al.* (1981) found N_2 fixation in microbial mats composed of non-heterocystous cyanobacteria. No information was given concerning the species composition of these mats, but specific mention was made of a *Lyngbya* sp. *Lyngbya aestuarii* has also been mentioned as a possible diazotroph in microbial mats (Potts, 1979; Carpenter *et al.*, 1978). The systematics of cyanobacteria remains problematic, and *Lyngbya* and *Oscillatoria* may easily be confused. It is therefore tempting to conclude that *Oscillatoria* spp. are particularly important as diazotrophs in many microbial mats. Although there is no doubt that *M. chthonoplastes* also contributes to N_2 fixation in some microbial mats, reported rates (Pearson *et al.*, 1979) were 10-100 times lower than those in mats in which *Oscillatoria limosa* fixed N_2 (Stal *et al.*, 1984).

On the wall of a limestone cave, Griffiths *et al.* (1987) discovered N_2-fixing populations of *Gloeothece*. Not much is known about the occurrence of this unicellular cyanobacterium in nature, but it has been found as a component of soil microbial communities (Smith *et al.*, 1990) and also occurs in rice fields (Huang & Chow, 1988). Although some species of *Gloeothece* are aquatic (Desikachary, 1959), there are no reports of marine strains of this cyanobacterium. In contrast, some strains of *Synechococcus* that have been studied are of marine origin (Duerr & Mitsui, 1980; León *et al.*, 1986; Spiller & Shanmugam, 1987), though others originate from the freshwater rice-field environment (Huang & Chow, 1988). However, there are no reports on the significance and ecology of N_2 fixation by non-heterocystous cyanobacteria in rice fields.

3.2 WHAT DIURNAL AND SEASONAL VARIATIONS OF N_2 FIXATION OCCUR IN COMMUNITIES OF NON-HETEROCYSTOUS CYANOBACTERIA?

The majority of cyanobacteria are obligate photoautotrophs, which implies that light is their ultimate source of energy. In nature however, light is present only during the day. Cyanobacteria therefore store part of the light energy, harvested during the day, in the carbon-reserve glucan, glycogen. Stored glycogen is metabolized during the night and provides the cell with a limited amount of energy in order to meet its maintenance requirements. However, some cyanobacteria may even be able to grow in the dark at the expense of endogenous glycogen (Post *et al.*, 1986). Glycogen, accumulated during the day, may therefore support processes that are energetically relatively expensive, such as growth and N_2 fixation, during the period of darkness.

Natural populations of heterocystous cyanobacteria show very distinct diurnal variations in N_2 fixation that are markedly dependent on light (Hübel & Hübel, 1974; Griffiths *et al.*, 1987; Aziz & Whitton, 1988). For instance, Vanderhoef *et al.* (1975) found that a bloom of *Aphanizomenon* sp. in Lake Mendota, Wisconsin, USA fixed N_2 mainly during the day. However, nitrogenase activities were highest during the

morning hours. A strong depression of nitrogenase activity at midday was also observed in a community of *Scytonema* sp. in mangrove forests in the Sinai (Potts, 1979), in microbial mats consisting mostly of *Calothrix contarenii* and on salt marsh sediments with *Stigonema* sp. as the dominant cyanobacterium (Carpenter *et al.*, 1978). During the night, natural populations of heterocystous cyanobacteria either fix no N_2 at all or fix N_2 at a decreased rate. Cultures of heterocystous cyanobacteria grown in the laboratory under alternating light and darkness also show light-dependence of nitrogenase activity, but the rate in the dark may be only partly decreased (Stal & Krumbein, 1985c; Griffiths *et al.*, 1987). In other cases an almost complete cessation of nitrogenase activity was observed during the dark phase (Mullineaux *et al.*, 1981). Thus, in heterocystous cyanobacteria, the ratio of nitrogenase activity in the light to that in the dark may vary among strains (Stal & Krumbein, 1985a). The midday depression in N_2 fixation that was observed by several investigators has been explained by a loss in buoyancy of planktonic organisms (Vanderhoef *et al.*, 1975) or, in benthic communities, by competition for electrons caused by photorespiration (Carpenter *et al.*, 1978). However, very recently, Storch *et al.* (1990) interpreted the different types of diurnal patterns of N_2 fixation in terms of a combination of CO_2 depletion and O_2 supersaturation. Samples with low biomass showed nitrogenase activity throughout the light period; this activity correlated well with light intensity. In the dark, activity in these samples was about 25% of that in the light. In contrast, nitrogenase activity in samples with high biomass showed the midday depression. Moreover, in these samples, no N_2 fixation was observed during the night. In samples with high biomass, supersaturation with O_2 and depletion of CO_2 was observed when the intensity of illumination was greatest, and the midday depression of nitrogenase activity could be prevented by sparging with N_2 and CO_2. The combination of O_2 supersaturation and CO_2 depletion would stimulate photorespiration (Bergman, 1981). Under such conditions, it is unlikely that any accumulation of glucan would occur. This in turn would explain the absence of nitrogenase activity during the night. In addition, inefficient CO_2 fixation in the light would limit the availability of organic acceptor molecules for assimilation of fixed nitrogen. The absence of carbon reserves may also have a severe impact on the ability of the cyanobacterium to protect nitrogenase from inactivation by O_2 (Ernst *et al.*, 1990). Finally, a high intracellular concentration of O_2 would favour photooxidative reactions and may cause photoinhibition, the disruption of photosynthetic electron transport itself (Kyle, 1987).

There are few reports concerning diurnal fluctuations of N_2 fixation in natural communities of non-heterocystous cyanobacteria, though, from laboratory studies, there is considerable evidence for a temporal separation between N_2 fixation and photosynthesis in these organisms. Almost all cultures of non-heterocystous cyanobacteria, unicellular as well as filamentous, fixed N_2 predominantly during the dark phase of a light:dark regime. A notable exception however is the marine planktonic cyanobacterium *Trichodesmium*. Nitrogenase activity in laboratory cultures of this organism was totally dependent on light (Ohki & Fujita, 1988). In addition, natural populations of *Trichodesmium* fixed N_2 exclusively during the light period (Saino &

Hattori, 1978; Bryceson & Fay, 1981; Capone *et al.*, 1990). Using chloramphenicol and immunochemical techniques, Capone *et al.* (1990) concluded that nitrogenase was synthesized *de novo* during the induction of activity at dawn and that inactivation of the enzyme, followed by degradation of the protein occurred at dusk. The involvement of *de novo* protein synthesis in the induction of nitrogenase during light:dark cycles was also shown by Stal & Krumbein (1985b) for the morphologically similar *Oscillatoria limosa*. This latter organism, however, induces nitrogenase synthesis in the dark. In contrast, with respect to its diurnal pattern of N_2 fixation, *Trichodesmium* behaves very similarly to heterocystous cyanobacteria. Furthermore, the O_2-sensitivity of *Trichodesmium* nitrogenase is comparable to that of the enzyme from heterocystous organisms.

In marine microbial mats on the sandy islands in the southern North Sea, *Oscillatoria limosa* is responsible for the fixation of N_2 (Stal *et al.*, 1984). This cyanobacterium is the dominant species during colonization of the intertidal sediments, but established mats are built by *Microcoleus chthonoplastes*, generally accompanied by 10-20% of *O. limosa*. Although one strain of *Microcoleus chthonoplastes* is known to fix N_2 aerobically (Pearson *et al.*, 1979), it is unlikely that this species contributed to N_2 fixation in these particular mats. For example, nitrogenase activity in the mats correlated well with the number of cells of *Oscillatoria limosa* and, in culture, *M. chthonoplastes* from the North Sea microbial mats was unable to fix N_2 under aerobic conditions. Moreover, a lag of 8h preceded the appearance of nitrogenase activity in *M. chthonoplastes* following transfer to nitrogen-free medium under anaerobic conditions (Stal & Krumbein 1985a). N_2 fixation in North Sea microbial mats showed both diurnal and seasonal fluctuations that, furthermore, varied with each location (Stal *et al.*, 1984, Villbrandt *et al.*, 1990). Although the absolute rate of N_2 fixation, expressed on a surface basis, was highest in established microbial mats, freshly colonized sand showed the highest specific rates of nitrogenase activity, when expressed on a chlorophyll *a* basis. Similarly, highest specific activities of nitrogenase in microbial mats were observed in spring, though highest absolute rates were seen in autumn. These observations are in agreement with the fact that a high demand for combined nitrogen accompanies both development of a microbial mat on nitrogen-depleted sand and reactivation of an existing mat in spring. Diurnal patterns of N_2 fixation were remarkably different at every site and season. Like many other non-heterocystous cyanobacteria, *O. limosa* fixes nitrogen mainly during the dark phase when grown under alternating light and darkness. However, in established mats, a transition from light to darkness is accompanied by a transition from oxic to anoxic conditions. Establishment of anoxic conditions occurs rapidly as a result of high respiratory activity. This activity arises not only from the considerable biomass of cyanobacteria but also as a consequence of the presence of other organisms in the mat. Although it is known that mat-building cyanobacteria are able to generate energy by fermentation of endogenous storage compounds (Heyer *et al.*, 1989) and that *Oscillatoria limosa* shows nitrogenase activity even under anoxic conditions in the dark (Stal & Heyer, 1987), it is evident that these conditions are far from optimal for N_2 fixation. Diurnal patterns of N_2 fixation in microbial mats are

therefore sometimes characterized by two peaks of activity (Stal *et al.*, 1984, Villbrandt *et al.*, 1990), one at sunset and one at sunrise. The activity peak at sunrise is usually especially pronounced. These diurnal patterns may be explained in terms of intracellular O_2 concentration, photosynthetic activity, light intensity and, perhaps, CO_2 concentration. During the night, N_2 fixation cannot take place because respiratory energy generation is impossible, a consequence of the absence of O_2. However, at dawn, light energy becomes available while that sediment is still anoxic and reducing conditions prevail. Such conditions would be particularly advantageous for N_2 fixation, and a peak of activity is seen. At dusk nitrogenase activity may be temporarily induced because oxygenic photosynthesis has ceased, O_2 supersaturation has disappeared but both O_2 and a low light intensity are available to support energy generation. Laboratory experiments in which *Oscillatoria limosa* was grown under a light:dark regime, with an accompanying change from oxic to anoxic conditions during the dark phase, showed exactly the same pattern of nitrogenase activity as that seen in microbial mats (Stal & Heyer, 1987). The same experiment was carried out independently with another strain of *Oscillatoria* and gave essentially the same results (Griffiths *et al.*, 1987). However, in its natural salt marsh habitat, this latter cyanobacterium showed a pattern of N_2 fixation characteristic of oxic light:dark grown cultures, with nitrogenase activity confined to the night. Griffiths *et al.* (1987) did not actually measure O_2 concentrations but, based on the behaviour of laboratory cultures, it seems likely that the natural populations of *Oscillatoria* were provided with O_2. This confinement of nitrogenase to the night period was also seen in freshly colonized intertidal sediments of the North Sea where the introduction of O_2 by diffusion from the atmosphere exceeds the low rates of respiration due to the small amount of biomass.

By comparing a great number of diurnal measurements of nitrogenase activity in microbial mats of North Sea intertidal sediments, it becomes clear that almost every possible pattern of N_2 fixation can be found. Bebout *et al.* (1987) reported similar variations among the diurnal patterns of N_2 fixation seen in North Carolina microbial mats. However, they found that nitrogenase activity always correlated inversely with CO_2 fixation. Environmental conditions such as temperature, light intensity and the frequency of flooding of the sediment, as well as the structure of the mat and its species composition, are all important and contribute to the observed pattern. These factors directly influence photosynthetic rates and the intracellular concentrations of O_2 and CO_2. Comparisons between different systems are therefore difficult, not only because of differences in experimental protocols but also because of differences in environmental conditions and species composition. As mentioned above, environmental conditions can influence the pattern of N_2 fixation seen in cultures of *Gloeothece* grown under alternating light and darkness. A pattern of N_2 fixation observed in the laboratory may not, therefore, always correspond to that found in natural populations.

3.3 HOW DO NON-HETEROCYSTOUS N_2-FIXING CYANOBACTERIA COPE WITH O_2 IN THE NATURAL ENVIRONMENT?

In order to avoid contact with O_2, N_2-fixing cyanobacteria might restrict themselves to anoxic environments. A considerable number of non-heterocystous cyanobacteria can fix N_2 only under anoxic or microaerobic conditions. The ecological importance of this behaviour is not well understood. The induction of this so-called 'anaerobic' nitrogenase activity is at least 3h but often exceeds this time. Therefore, in environments that experience frequent changes from oxic to anoxic conditions, anaerobic N_2-fixing cyanobacteria would probably be unable to grow diazotrophically. Environments that stay anoxic for a long period seem to be more suitable for anaerobic N_2 fixation. However, there are only a few examples of such environments that are dominated by cyanobacteria. These include the hypolimnion of Solar Lake, Sinai, which, because it contains high concentrations of sulphide, is anoxic during most of the year. This habitat is dominated by *Oscillatoria limnetica*, which is capable of very efficient anoxygenic growth at high concentrations of sulphide. *Oscillatoria limnetica* is capable of anaerobic N_2 fixation (Padan & Cohen, 1982) but, to our knowledge, this has never been shown in its natural environment.

In microbial mats it has been shown that the specific nitrogenase activity (expressed on a chlorophyll *a* basis) increased with depth within the cyanobacterial layer (Pearson *et al.*, 1979; Stal *et al.*, 1984). Highest specific activity was found in the layer 2-3 mm below the surface. Using microelectrodes, it was shown that cyanobacteria in this layer did not carry out oxygenic photosynthesis and that O_2 was totally absent. Nevertheless, far red light could penetrate this layer and probably supported both nitrogenase activity and anoxygenic photosynthesis. These observations could be taken to support a spatial separation of oxygenic photosynthesis (in the upper layers) and N_2 fixation (in the lower layers). However, separating N_2 fixation and oxygenic photosynthesis in this manner would imply either a moving up and down of cyanobacterial filaments in the sediment or a transfer of nutrients between layers. Neither has so far been demonstrated in marine microbial mats.

Another option is the confinement of N_2-fixing cyanobacteria to O_2-poor microzones in an otherwise oxic environment. Paerl & Prufert (1987) have shown that O_2-depleted microniches develop in microbial aggregates in marine waters. The enrichment of these aggregates by metabolizable organic matter was necessary for the removal of O_2 as well as for fixation of N_2. In the water column, O_2-poor microzones are likely to be associated exclusively with particles. On the contrary, in sediments, anoxic microniches are ubiquitous and Paerl *et al.* (1989a) suggested that they might be the sites of N_2 fixation in microbial mats dominated by non-heterocystous cyanobacteria (*Lyngbya aestuarii, Microcoleus chthonoplastes, Oscillatoria* spp., and *Phormidium* spp.). Several reports have emphasized the role of the formation of aggregates for N_2 fixation by non-heterocystous cyanobacteria (Carpenter & Price, 1976; Carpenter & Walsby, 1979; Bryceson & Fay, 1981; Bautista & Paerl, 1985; Paerl *et al.*, 1989b). In the case of *Trichodesmium*, it had been assumed for some time that the central region of the bundles of trichomes provides an O_2-depleted

environment in which nitrogenase can flourish (for a summary of the studies supporting this view, see Carpenter *et al.*, 1990). However, laboratory studies did not support this view (Ohki & Fujita, 1988) and immunochemical localization of nitrogenase in aggregates of *Trichodesmium* showed nitrogenase to be present in at least some cells throughout the colony (Paerl *et al.*, 1989c; Bergman & Carpenter, 1991). Furthermore, a recent detailed examination of natural populations showed that cells in the central regions of colonies of *Trichodesmium* had a similar composition of photosynthetic pigments to that of cells at the edge of colonies. Central cells may therefore photoevolve O_2. In addition, the centre of colonies was probably not anoxic (Paerl *et al.*, 1989b), and the measured rate of N_2 fixation was unaffected by colony size (Carpenter *et al.*, 1990). Clumping of cells into colonies may not therefore, of itself, be important in explaining aerobic N_2 fixation in *Trichodesmium*. The same is true of clumps of *Microcoleus chthonoplastes* (Malin & Pearson, 1988) and, perhaps, also of certain *Oscillatoria* spp (Gallon *et al.*, 1991b). Nevertheless, disruption of clumps of *Trichodesmium* by, for example, wave action markedly decreased the rate of N_2 fixation (Carpenter & Price, 1976; Bryceson & Fay, 1981). Carpenter *et al.* (1990) have therefore concluded that, as in other diazotrophs, no single mechanism protected nitrogenase from the deleterious effects of atmospheric O_2 but that at least one mechanism was affected by the degree of aggregation of bundles of filaments.

Association with heterotrophic bacteria may be beneficial for N_2-fixing cyanobacteria, as shown for *Anabaena* (Paerl, 1978; Paerl & Kellar, 1978). Heterotrophic bacteria preferentially associate with the heterocysts of *Anabaena* and they are thought to decrease the local concentration of O_2. The non-heterocystous, microaerophilic cyanobacterium, *Plectonema boryanum*, grows diazotrophically very well under photoautotrophic conditions provided that the prevailing concentration of O_2 is kept low (Pearson & Howsley, 1980; Giani & Krumbein, 1986). In nature, such cyanobacteria are likely to be associated with heterotrophic bacteria, which might decrease the local concentration of O_2 sufficiently to allow N_2 fixation. However, no such N_2-fixing communities appear to have been studied. It would be interesting to know for example whether, under alternating light and darkness, anoxic populations of microaerophilic non-heterocystous cyanobacteria fix N_2 only in the light (like microaerobically grown cultures of *Gloeothece*), only in the dark (supporting N_2 fixation by anaerobic metabolism), or both in the light and the dark. In sediment ecosystems or in stagnant paddy fields O_2 would be expected to accumulate in the light, and the photic zone of such systems (notably in the immediate surroundings of any cyanobacteria) would become supersaturated with O_2. In the dark, this situation would be reversed: O_2 would be rapidly consumed and the environment would become anoxic. Neither situation is ideal for N_2 fixation, and diazotrophs that live in such environments would need to be well-adapted to these fluctuating conditions. However, in non-stagnant aquatic environments (notably the open ocean) sub- and supersaturation with O_2 would be less common.

3.4 WHAT ARE THE POSSIBLE ECOLOGICAL ADVANTAGES FOR NON-HETEROCYSTOUS CYANOBACTERIA OVER HETEROCYSTOUS SPECIES?

There is probably no answer to this intriguing question, but a consideration of this subject may give directions for future research.

To the ecologist, one of the most striking differences between heterocystous and non-heterocystous cyanobacteria is the habitat in which they occur. Only a few marine heterocystous cyanobacteria are known. *Calothrix* spp. sometimes form an 'algal crust' type of microbial mat in hypersaline and intertidal environments (Javor & Castenholz, 1981), and a few other heterocystous species have been isolated from marine environments. However, as mentioned earlier in this article, enrichment for aerotolerant N_2-fixing cyanobacteria almost always results in the isolation of heterocystous species and these isolates can usually be considered not as truly marine but rather as salt-tolerant (Stal & Krumbein, 1985a). Furthermore, with the notable exception of the benthic *Calothrix* spp., heterocystous cyanobacteria probably make only a minor quantitative contribution to marine N_2 fixation. On the other hand, in freshwater and terrestrial environments, heterocystous species seem to be the predominant N_2-fixing cyanobacteria. An exception may be the *Gloeothece*-dominated walls of limestone caves, where the prevailing intensity of illumination is low. Significantly, near the entrance of one such cave, where the level of illumination was greater, *Nostoc* sp. was the dominant organism (Griffiths *et al.*, 1987). Similarly, rice fields contain mainly heterocystous cyanobacteria, though some unicellular species have been isolated from this environment (Roger *et al.*, 1986; Huang & Chow, 1988).

Besides the marine/freshwater contradistinction, differences in the maintenance requirements and optimum light intensity may play an important role in determining the distribution of heterocystous and non-heterocystous cyanobacteria. For example, N_2-fixing heterocystous cyanobacteria usually need high irradiances and have a relatively high maintenance requirement (Zevenboom & Mur, 1980). This can be explained in terms of the high energy demand of nitrogenase and the fact that only 5-7% of cells differentiate into heterocysts, yet have to provide the remaining 93-95% of vegetative cells with combined nitrogen.

Cell differentiation leads to specialization. Heterocystous cyanobacteria are highly specialized prokaryotes, perfectly adapted to live without combined nitrogen. However, specialization results in loss of versatility and metabolic flexibility. Consequently, in environments that experience large fluctuations in prevailing conditions, flexible and versatile organisms will have an advantage. It is, perhaps, in just such environments that non-heterocystous N_2-fixing cyanobacteria are able to outcompete heterocystous species.

4. Concluding remarks

If, as T.H. Huxley has remarked, the tragedy of science is "a beautiful hypothesis slain by an ugly little fact", then those of us who study N_2 fixation in cyanobacteria have seen our fair share of misfortune. For example, the idea that all N_2-fixing cyanobacteria possess heterocysts is no longer with us, nor do we now hold with the view that non-heterocystous cyanobacteria can fix N_2 only anaerobically or microaerobically. Is another hypothesis, namely that natural populations of non-heterocystous diazotrophic cyanobacteria effect either a spatial or a temporal separation between N_2 fixation and photosynthetic O_2 evolution, about to bite the dust? That remains to be seen, but its future seems to rest squarely with *Trichodesmium*. We must therefore await detailed studies on this fascinating organism. Incidentally, anyone who is offended by the idea of *Trichodesmium* as an 'ugly little fact' might take comfort in the thought that it would not be unique in this respect. Both *Plectonema boryanum* and *Gloeothece* were the 'ugly little facts' of an earlier day (Gallon & Chaplin, 1988a).

However, hypotheses have their place in scientific research in that they act as a spur to further investigations. Thus, although most of the laboratory studies on non-heterocystous N_2-fixing cyanobacteria have used organisms that seem to behave differently from planktonic marine cyanobacteria, the findings from these studies have undoubtedly influenced work with *Trichodesmium*. For example, researchers are now looking at diurnal fluctuations of nitrogenase synthesis as well as activity in *Trichodesmium* and at whether any of the strategies for handling O_2 found in other non-heterocystous cyanobacteria might occur in this organism. In addition, using immunological techniques devised during laboratory studies on other cyanobacteria, the location of nitrogenase has been probed, as has how nitrogenase might be subject to modification in natural populations of *Trichodesmium*. It will probably not be long before work with *Trichodesmium* and other planktonic marine cyanobacteria influences laboratory work with other species.

It is clear that *Trichodesmium* is different from most N_2-fixing non-heterocystous cyanobacteria. In particular, N_2 fixation in *Trichodesmium* is confined to the light period of a cycle of alternating light and darkness. Once the mechanism underlying this behaviour emerges, our thinking about how non-heterocystous cyanobacteria behave may be fundamentally altered. Meanwhile, this finding raises new questions. For example, why does *Trichodesmium* fix N_2 only in the light? This behaviour is seen in many heterocystous cyanobacteria (see, for example, Khamees *et al.*, 1987): is there a common underlying mechanism? Perhaps so, but perhaps not. It is worth remembering that, at the molecular level there appear to be differences in the way in which other non-heterocystous cyanobacteria confine N_2 fixation to the dark period of a light:dark cycle, even though the overall pattern of behaviour is the same. Moreover, cultures of *Gloeothece* incubated microaerobically, or in continuous culture, fix N_2 during the light phase of an alternating cycle of light and darkness. Are there any parallels here with the behaviour of *Trichodesmium*? We might also

ask whether there is an intercellular (intertrichome) spatial separation between N_2 fixation and photosynthesis in *Trichodesmium*. If so, what fluxes of nutrients exist between the two different types of cell? If not, how does nitrogenase survive within an O_2-evolving cell? Here the problems may be the same as in other non-heterocystous cyanobacteria incubated under continuous illumination though it is still not clear in these organisms whether individual cells ever simultaneously fix N_2 and photoevolve O_2. At present we do not know how *Trichodesmium* behaves under constant illumination. Hopefully, answers to these questions will be easily obtained once we have consistently good laboratory cultures of *Trichodesmium*. Therein lies yet another question, why is culture so difficult?

This chapter has been full of questions. Some, we have attempted to answer; others we have raised merely in an attempt to point some of the ways forward. These questions are by no means exhaustive and it is not too difficult to think of others. However, it is appropriate that this book is being prepared at a time when interest in N_2 fixation in the marine environment is particularly acute and when rapid progress is being made. As a consequence, many of the questions posed may have been answered by the time these words are published. However, if so, we can be confident that at least an equal number of new questions will have arisen. We live in exciting times!

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THE GAS VESICLES AND BUOYANCY OF *TRICHODESMIUM*

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ABSTRACT. Gas vesicles provide *Trichodesmium* colonies with buoyancy. When the sea is calm the colonies float up and accumulate at the surface where they will be conspicuous to remote sensors. Under rough conditions they are mixed down, sometimes to great depths, but they may float back rapidly by virtue of their low density and large size. *Trichodesmium* gas vesicles are narrower, and therefore less efficient in providing buoyancy, than those of other cyanobacteria, but this gives them greater strength. They therefore withstand pressures generated at greater depths without collapse. *Trichodesmium* colonies regulate their buoyancy in response to irradiance during the diel cycle.

1. Introduction

The first reports of *Trichodesmium* floating at the ocean surface are probably those of Joseph Banks who recorded in his *Endeavour Journal* of 1770 "vast quantities of the little substances...floating upon the water in large lines a mile or so long...either immediately upon the surface or not many inches under it. The seamen began to call it Sea sawdust". Embodied in these few lines are a number of significant features of *Trichodesmium*: it forms particles visible to the naked eye; it floats; it accumulates in the convergent downwellings of Langmuir circulation cells; and it forms extensive surface waterblooms. From these few threads we can construct part of the biology of this organism. First the story and then the details.

Trichodesmium (which means "a bundle of threads") is a colonial, filamentous cyanobacterium. The filaments aggregate either parallel to one another to form fusiform colonies or radially to form spherical colonies that may be a millimetre or more across. These large colonies are buoyant and float up at velocities often exceeding 1 mm s^{-1}, or 90 metres per day. Their buoyancy is provided by gas vesicles, which, there is reason to believe, are especially adapted to withstand the pressures that these organisms experience when they are swept down to great depths during storms. If their gas vesicles are not collapsed the organisms remain buoyant and float back up to the water

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surface. The speed at which they float depends on the colony size; the large colonies float much faster than small ones and arrive sooner back in the illuminated surface layers. By remaining buoyant they spend more time in the euphotic zone performing photosynthesis, and this may enable them to outcompete other phytoplankton.

The ability to float permits the colonial habit for a phytoplankton organism; without buoyancy an increase in colony size gives a much increased sinking rate and leads to loss from the euphotic zone. For the floater, the colony may provide other advantages. Cells in the colony may benefit from periodic self-shading in the intense surface illumination. The colony may be large enough to sustain gradients of gas diffusion that permit anaerobic regions to form, and allow nitrogen fixation to occur. The colonial form may also act as a deterrent to grazing. Its prime significance, however, must be related to flotation. In freshwater habitats, as well as marine, all the colonial cyanobacteria are gas-vacuolate ones that can become buoyant.

The ability of *Trichodesmium* to float explains how it can produce such concentrated populations at the water surface. The biomass in these populations may be locally very high, but averaged over large areas it may not necessarily be higher than that of other phytoplankton in the underlying water column. Its abundance at the surface may therefore be illusory to the observer above it. This is particularly pertinent in remote sensing where the radiation reflected from the phytoplankton may penetrate only a few centimetres of water. Nevertheless, in the tropical ocean, where the thermal stability of the water column often allows a floating organism to rise, *Trichodesmium* can form massive populations which must incorporate significant quantities of carbon and nitrogen from the atmosphere and make an important contribution to the productivity of the ocean.

In the following discussion of gas vesicles and buoyancy I first summarise the many investigations that have been made on other, mainly freshwater, cyanobacteria. This then provides a platform for the discussion of *Trichodesmium*, which has been little investigated.

2. Gas Vesicles

2.1. GAS VESICLES IN CYANOBACTERIA

Gas vesicles are hollow cylindrical structures with cone-shaped ends (Bowen and Jensen 1965; see Walsby (1972) for a review). Both the cylinders and cones have a regular ribbed construction (Jost 1965). The dimensions of gas vesicles vary in different species. Their width, or more precisely cylinder diameter, is fairly uniform in each species and

ranges from about 60 to 110 nm (Walsby and Bleything 1988). There is a larger range of length, because the gas vesicle grows from a small biconical initial to the mature cylindrical form (Waaland and Branton 1969; Lehmann and Jost 1971) which may exceed 1000 nm in length, but the average length is in the range of 300 to 700 nm (Walsby and Bleything 1988). The wall of the gas vesicle, which is made of protein, is 1.8 nm thick in *Anabaena* (Blaurock and Walsby 1976), and probably the same in other organisms. From the geometry, dimensions and density of the wall protein, 1330 kg m^{-3}, it is possible to calculate the overall density of the gas vesicle; it varies from 100 to 162 kg m^{-3}, or between a tenth and a sixth of that of water. There is no solid or liquid material that has such a low density, and the gas vesicle is therefore an effective provider of buoyancy. A gas bubble has an even lower density but bubbles small enough to fit inside cyanobacterial cells have such a high pressure generated on them by surface tension that they are unstable.

Inside cyanobacterial cells gas vesicles form aggregates called gas vacuoles, which in the light microscope appear as bright refractile granules. In the gas vacuole the cylindrical gas vesicles are stacked together in hexagonal arrays, like the cells of a honeycomb (Jost 1965). Sometimes the gas vacuoles occupy distinctive positions at the cell periphery (Waaland et al. 1971) or at the septa that separate adjacent cells in the filament (Whitton and Peat 1969).

2.1.1. *Physical properties.* The gas vesicle is rigid, permeable to gases, and impermeable to liquid water. Pressures on the outside of the structure are not transmitted to the gas inside but are borne by the stiffness of the protein wall. The *Anabaena* gas vesicle has a compressibility of only 0.00155 bar^{-1} i.e., it shrinks by only one part in 650 when the pressure is increased by 1 bar (Walsby 1982). The *Microcystis* gas vesicle, which is narrower, shrinks even less, by one part in 1150 (Walsby 1991). This low compressibility means that little of the buoyancy provided by gas vesicles is lost in cells that are carried to greater depths.

The gas vesicle wall is permeable to gases (Walsby 1969). Gases dissolved in the suspending water rapidly diffuse in and out of the structure, equilibrating in less than a millisecond (Walsby 1984). Consequently the gas inside the gas vesicle is usually air at atmospheric pressure, like that dissolved in water. Although the gas diffuses extremely rapidly across the structure, a gas vesicle suspension conducts gas slightly more slowly than an equivalent layer of water (Walsby and Revsbech, 1990) and it may be doubted, therefore, whether randomly oriented gas

vesicles can act as useful gas conducting channels (but see section 2.2.3.).

The inner surface of the gas vesicle is hydrophobic and this prevents liquid water from entering. The outer surface, in contrast, is hydrophilic; this minimizes the interfacial tension on the structure, which might otherwise tend to cause its collapse (Walsby 1972).

2.1.2. *Relationship between critical pressure and diameter.* Although moderate pressures cause little compression of the gas vesicle, application of pressures above a certain threshold, known as the critical pressure (p_c), cause sudden, irreversible collapse. The cylindrical structure is flattened to a rectangular envelope and the conical ends flatten and tear away. This indicates the engineer's second mode of collapse by instability failure, following buckling, which is typical of rigid thin-walled structures. For structures of a given wall thickness the critical pressure varies inversely as a function of the cylinder radius (r). Walsby and Bleything (1988) investigated this relationship between critical and diameter of gas vesicles in 7 genera of cyanobacteria and found the expected inverse variation, described by the equation

$$p_c = 275(r/\mathrm{nm})^{-1.67}\ \mathrm{MPa}. \qquad (1)$$

Thus the mechanics of the engineer's pressurised cylindrical tube are applicable at the macromolecular scale of the gas vesicle.

2.1.3 *Natural selection for width and strength.* What lies behind the variation in size and strength of the gas vesicle is the need to withstand different pressures in different environments. At one extreme, the gas vesicles in the cyanobacterium *Dactylococcopsis salina* from Solar Lake in the Sinai Peninsula (Walsby et al. 1983a) have to withstand the combination of a small hydrostatic pressure in the lake and a low turgor pressure in the halophilic cells (see Fig. 1). An average critical pressure of about 3 bars suffices, and this can be provided with a wide gas vesicle of radius 55 nm. At the other extreme of the examples investigated by Walsby and Blething (1988) were the narrower gas vesicles in a red-coloured strain of *Oscillatoria aghardii* from Lake Gjersjoen, a deep lake in Norway (Walsby et al. 1983b), where the much higher hydrostatic pressure at holomixis and the greater turgor of these freshwater cells require gas vesicles with an average critical pressure of 10 bar (Fig. 1), which requires a much narrower structure, of radius 30 nm. These narrow structures provide less buoyancy for a given investment of gas vesicle wall material. Between these extremes were intermediate forms with gas vesicles

adapted to intermediate pressures. As indicated below, the gas vesicles of *Trichodesmium* are narrower and stronger than those of any other cyanobacteria yet examined, and this can be explained by the natural selection of forms that withstand much greater pressures in the oceans.

2.1.4. *Gas vesicle proteins.* Gas vesicles are constructed entirely of protein. The properties of the gas vesicle must therefore be provided by the gas vesicle protein; the differences between the gas vesicles of different species must be due to differences in these proteins or in other factors involved in the assembly or modification of the structures. For a review on gas vesicle proteins see Walsby and Hayes (1989); a brief summary is given here.

There are two types of gas vesicle protein (GVP). The main structure of the gas vesicle is formed by GVPa, a small hydrophobic protein of 70 amino acid residues in *Anabaena* (Walker et al. 1984), and *Calothrix* (Tandeau de Marsac et al. 1985). GVPa proteins with highly homologous N-terminal sequences have been found in 8 genera of cyanobacteria (Walker et al. 1984; A.E. Griffiths and A.E. Walsby, in preparation). This protein is arrange in a crystalline fashion in the 4.6 nm-wide ribs, which form both the cylinder and cones of the gas vesicle. The size of the protein, 7.4 kDa, corresponds to the size of the crystalline unit cell that repeats along the ribs (Blaurock and Walsby 1976).

The second protein, GVPc, is larger, usually in the range of 19 to 22 kDa, and hydrophilic in character (Damerval et al. 1987; Hayes et al. 1988). A striking feature of its primary structure is that it contains high-homologous repeats of 33 amino acid residues; there are 4 of these in the *Calothrix* GVPc (Damerval et al. 1987) and 5 in *Anabaena* GVPc (Hayes et al. 1988). In *Anabaena* gas vesicles the GVPa forms 92% of the mass and the GVPc the remaining 8% (Hayes et al. 1988). The GVPc must be on the outside of the structure because it can be removed from them, by rinsing with detergent. The gas vesicles are not collapsed by this treatment but they do get much weaker, suggesting that GVPc helps to support and stiffen the structure (Walsby and Hayes 1988). We have recently shown that GVPc can be reassembled onto the gas vesicles from which it had been removed, and that this restores their strength to nearly its original value (P.K. Hayes, B. Buchholz and A.E. Walsby, submitted). We have speculated that the 33-amino acid repeats in the GVPc form 5 nm long alpha-helices, which cross the GVPa ribs and bind them together (Hayes et al., 1988)

2.2. GAS VESICLES IN *TRICHODESMIUM*

It was known for many years that gas vacuoles occurred and

provided buoyancy in numerous species of freshwater cyanobacteria (see, for example, Klebahn 1895, 1922; and Geitler 1932). Despite knowledge of its floating habit (Geitler 1932), there was a long delay before gas vacuoles were noticed in *Trichodesmium* (Margalef 1965). The probable reason for this was that the identity of the gas vacuoles could not be confirmed by their disappearance in the "hammer, cork and bottle" experiment of Klebahn (1895) because they were too strong to be destroyed by this method (see Walsby 1978).

2.2.1. *Fine structure.* Van Baalen and Brown (1969) showed that in *Trichodesmium erythraeum* gas vacuoles formed cylindrical structures within the cytoplasm. The electron micrographs indicated two concentric layers of hexagonally stacked gas vesicles with their long axes oriented radially in the cell (i.e., like the spokes of a wheel). The diameter of the individual gas vesicles was about 78 nm. Another detailed study was made of gas vacuole fine structure in *Trichodesmium thiebautii*, by Gantt, Ohki and Fujita (1984). In this species the gas vesicles were also arranged in two layers at the cell periphery, though not in quite such a uniform cylinder. The gas vesicle were much narrower, only 45 nm in diameter.

In the spaces between the gas vesicles were electron dense fibres of 25 nm diameter. Although they were dispersed throughout the gas vacuoles they did not have a uniform association with the constituent gas vesicles. There were about 3 times as many gas vesicles as fibres. Many of the fibres were in pairs. Their composition and function is unknown. Borstad and Borstad (1984) observed other needle-like bodies, 0.2 to 0.5 m wide, issuing from the autolysing cells of *Trichodesmium hildenbrandtii*. The needles were said to float.

2.2.2. *Critical pressures.* The gas vesicles in *Trichodesmium* are extremely strong. Their median critical pressures are in the region of 1.2 MPa in *T. erythraeum*, 3.4 MPa in *T. contortum*, and 3.7 MPa in *T. thiebautii* (Walsby 1978). These measurements were made with the gas vesicles inside turgid cells suspended in seawater so that the actual critical pressures must be greater, by an amount equal to the cell turgor pressure. The magnitude of this in marine cyanobacteria is unknown but it may be in the order of 0.1 MPa.

If these gas vesicles showed the same relationship between p_c and r described by equation (1) then those of *T. erythraeum*, would be expected to have a diameter of 53 nm (instead of the 78 nm found by van Baalen and Brown 1969), and those of *T. thiebautii* would have a diameter of 28 nm (instead of the 45 nm found by Gantt et al. 1984). At

present, no definite conclusions can be drawn about the relationship between the width and strength of the *Trichodesmium* gas vesicles because the measurements were made on different populations; simultaneous investigations are now needed. The dimensions of cell structures cannot be determined with great accuracy from thin-sectioned material, which may undergo volumetric changes during fixation and embedding. The gas vesicles should be isolated and dried onto mica for subsequent analysis by shadowing techniques (Jost and Jones 1970; Walsby and Blething 1988). Preparations of isolated gas vesicles would also be valuable for analysis of the gas vesicle proteins. It would be interesting to see if there were additional proteins that increased the thickness and strength of these exceptionally strong gas vesicles.

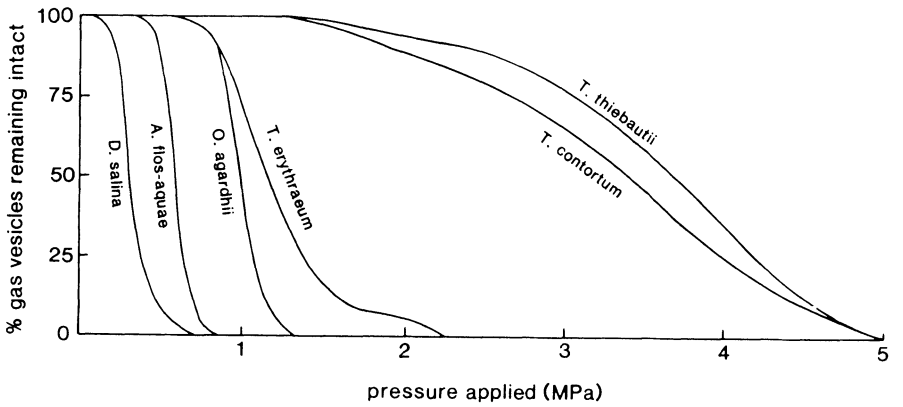

Fig. 1. Critical pressure distributions of the gas vesicles in the cells of three species of *Trichodesmium*, suspended in seawater (data from Walsby 1978), and in the cells of the freshwater cyanobacteria *Oscillatoria agardhii* and *Aphanizomenon flos-aquae*, and of the halophilic cyanobacterium *Dactylococcopsis salina*, suspended in hypertonic sucrose solutions to remove cell turgor pressure (data from Walsby and Bleything 1988).

It is clear that the gas vesicles of great strength are needed in *Trichodesmium* if the colonies deep in the ocean are to retain or regain their buoyancy.

2.2.3. *Other functions of gas vesicles?* The buoyancy providing function of gas vesicles is in no doubt, but are there other functions also? Two have been suggested.

Van Baalen and Brown (1969) observed that most of the thylakoids in the cells lay within the peripheral layer of gas vesicles. This led them to suggest that the gas vesicles might shade the photosynthetic structures by interference effects or internal reflection. These ideas have not been rigourously tested. When *Nostoc muscorum* is grown at high irradiance its gas vesicles are also oriented at the cell periphery and spectrophotometric data suggests that some shading of the photosynthetic pigments occurs (Waaland, Waaland and Branton 1971). The same peripheral orientation occurs in *Anabaena flos-aquae* at high irradiance (Shear and Walsby 1975) but in this organism collapsing the gas vesicles gave no significant increase in photosynthetic rate, and there was therefore no evidence of light shielding. The question of light shielding as a secondary function in *Trichodesmium* is open, and requires direct investigation.

With the discovery that gas vesicles were permeable to gases (Walsby 1969), Taylor, Lee and Bunt (1973) made the suggestion that oxygen formed by the photosynthetic apparatus located in the inner cytoplasm might diffuse outwards into the gas vacuoles. They suggested that this might provide sufficiently microaerobic conditions for nitrogen fixation to occur, though it was not quite clear how it would do so. The gas permeability of a randomly oriented layer of *Anabaena* gas vesicles is a little less that of water (Walsby and Revsbech 1990). A similar layer of the narrower *Trichodesmium* gas vesicles would be still less permeable though a layer of such gas vesicles oriented with their long axes parallel to a gas concentration gradient could provide a gas diffusion channel (Walsby, Revsbech and Griffel, in preparation). For *Trichodesmium*, where they are oriented radially, the gas diffusion channel would therefore be across the cell rather than along the filament. This should be considered in discussing the oxygen diffusion gradients and nitrogen fixation in *Trichodesmium* colonies (Fogg 1974; Carpenter and Price 1976; Bryceson and Fay 1981).

3. Buoyancy

3.1. BUOYANCY IN CYANOBACTERIA

Cells of cyanobacteria, and other phytoplankton, will float only if their density is less than that of the water in which they are suspended. The density of freshwater at 20°C is about 998 kg m^{-3} and that of seawater 1028 kg m^{-3}. Protein, carbohydrate and most of the other principal constituents of most cytoplasm, are considerably denser than both freshwater and seawater, and the majority of cells therefore sink. Some aquatic organisms become positively

buoyant by accumulating substances of low density (for reviews see Smayda, 1967 and Walsby and Reynolds 1981). In cyanobacteria buoyancy is provided by gas vesicles.

3.1.1. *Floating and sinking velocities.* A small spherical object, of radius r and density $\underline{r}$, sinks in water, of density $\underline{r}'$ and viscosity $\underline{h}$, at a velocity u given by the Stokes equation:

$$u = 2gr^2(\underline{r} - \underline{r}')/9\underline{h} \qquad (2)$$

where g is the gravitational acceleration. From this it follows that large colonies of cells sink much faster than individual cells of the same density. Colonies always have a large proportion of interstitial space, however, which is occupied either by water or by mucilage of nearly the same density as water, and the excess density of the colony ($\underline{r} - \underline{r}'$) is therefore usually much less than that of the cell. In practice r^2 dominates and colonies do sink much faster. Thus while single cells or filaments rarely sink faster than 10 m s^{-1}, colonies may sink at velocities exceeding 1 mm s^{-1} (see Booker and Walsby 1979, Walsby et al, 1983b; Walsby and MacAllister 1987).

When $\underline{r} < \underline{r}'$, the excess density is negative; then the negative of u is the floating velocity. Cells or colonies with very high gas vesicle content may have a floating velocity that equals or even exceeds the sinking velocity when gas vesicles are subsequently collapsed.

3.1.2. *Buoyancy regulation.* Studies on freshwater cyanobacteria have shown that they can regulate their buoyancy in response to light: they become more buoyant at low irradiance and lose buoyancy at high irradiance (Walsby 1969). The loss of buoyancy can be caused by the loss of gas vesicles or increase in dense substances such as carbohydrate (Oliver et al. 1982).

In *Anabaena*, gas vesicles can be lost by collapse under rising turgor pressure at high irradiance (Dinsdale and Walsby 1972; Oliver and Walsby 1984). More than 50% of the gas vesicles may be lost when the turgor rises by as much as 0.24 MPa to 0.54 MPa (Kinsman et al. 1991). In certain other cyanobacteria, such as species of *Oscillatoria*, gas vesicles are too strong for collapse by turgor pressure to occur (Walsby et al. 1983b).

To find the cause of buoyancy loss it is necessary to draw up a balance sheet of the ballast masses of the major cell constituents, before and after exposure to high irradiance (Oliver and Walsby 1984). In this way it has been demonstrated that *Oscillatoria* filaments lost buoyancy by increasing their protein and carbohydrate content and ceasing to make enough new gas vesicles to compensate

(Utkilen et al., 1985). Buoyancy loss in *Microcystis* was accounted for entirely by carbohydrate increase. Even in cyanobacteria, such as *Aphanizomenon* and *Anabaena*, capable of collapsing gas vesicles by turgor rise, buoyancy may be lost first by carbohydrate increase (Konopka, et al. 1987a and b; Walsby et al. 1989; Kinsman et al. 1991). There are other accounts of buoyancy loss being correlated with carbohydrate increase at high light intensity (Kromkamp and Mur 1984; Van Rijn and Shilo 1985). Since carbohydrate is produced universally in photosynthesis, its accumulation at high irradiance may be a common cause of density increase.

Both turgor rise and carbohydrate accumulation occur when the rate at which carbon is assimilated by photosynthesis exceeds the rate at which it is incorporated into various cell constituents. It is not surprising, therefore, that light-mediated buoyancy regulation is also affected by the availability of carbon dioxide, combined nitrogen and phosphate (Dinsdale and Walsby 1972; Klemer 1978, Spencer and King 1985; Konopka et al. 1987a and b).

3.1.3. *Buoyancy regulation and vertical migration.* In natural waters the irradiance decreases exponentially with depth. A buoyant cyanobacterium floating up towards the surface during daylight will encounter an increasing irradiance and, by the mechanisms described above, will tend to lose its buoyancy and then sink. As it sinks down into lower irradiance the process is reversed. In this way the cyanobacterium will exhibit cycles of vertical migration. The periodicity of these cycles and the depth over which migration occurs is determined by the rate coefficients of density change, the sinking or floating velocity, and the interactions with the changing illumination at the water surface. These interactions have been quantitatively described by computer models (Walsby 1988; Kromkamp and Walsby 1990) and are discussed in more detail in this volume by Kromkamp. These models explain why small separate filaments tend to stratify in the metalimnion (Baker and Brook 1969; Brook et al. 1971; Walsby and Klemer 1974; Walsby et al. 1983b) while large colonial forms migrate over many metres, from the surface to the hypolimnion during the day, and back to the surface at night (Walsby and MacAllister 1987; Ibelings et al. 1991a).

3.1.4. *Waterblooms.* Under calm conditions buoyant cyanobacteria may accumulate at the surface of lakes (Reynolds 1971) and the oceans (Bainbridge 1957; Ferguson Wood 1965; Fogg 1974) forming waterblooms. The process has been studied most extensively in lakes (for reviews see Reynolds and Walsby 1975; Reynolds 1987; Paerl 1988).

The potential advantages to cyanobacteria of occupying the water surface are that (1) they receive high irradiance

for photosynthesis, (2) they have access to carbon dioxide entering the water from the overlying atmosphere (Walsby 1970), and (3) the surface may become enriched with combined nitrogen and other nutrients (Lewis 1983).

The water surface may hold disadvantages, however. (1) Prolonged exposure to high irradiance may result in photooxidation, to which cyanobacteria may have different degrees of resistance (Abelievich and Shilo (1972). (2) The rate at which carbon dioxide invades from the atmosphere may be insufficient to maintain adequate concentrations in water containing high concentrations of cyanobacteria consuming the gas (Booker and Walsby 1981). (3) Nitrate, phosphate and other nutrients may similarly become exhausted by the concentrated populations. On balance the disadvantages of remaining at the water surface are likely to outweigh the advantages of being there.

Probably, the main selective advantage to cyanobacteria in forming large, buoyant, fast-floating colonies is not to form waterblooms **at** the surface *per se* but to maintain themselves **near** the surface in the euphotic zone. This conclusion is supported by the quantitative analyses of Humphries and Lyne (1988). After mixing events the buoyant colonies return rapidly to the surface layer and they become distributed towards the more productive surface layers during periods of calm. In this way they spend more time performing photosynthesis and they shade out potential competitors that are distributed throughout the surface mixed layer (Ibelings et al. 1991b). Surface waterbloom formation is, then, a consequence of this buoyancy that occurs under very calm conditions. Overall, the cyanobacteria must gain more from the chance of remaining near the surface than they lose from the chance of perishing at the surface. That is, anyway, the conclusion to be drawn from the success of buoyant colonial cyanobacteria.

Buoyancy regulation provides a way of minimising the time spent at the water surface itself. It is apparent, however, from the persistence of cyanobacteria at the water surface that their methods of escaping by losing buoyancy at high irradiance are not always effective. This failure, when it occurs, is perhaps explained by one of the following factors.

(1) The rate of carbon dioxide arrival may become rate-limiting to photosynthesis in the concentrated surface population (Booker and Walsby 1981). Carbon dioxide is necesary for buoyancy loss by turgor rise (Dinsdale and Walsby 1972) and carbohydrate accumulation. There is evidence that carbon dioxide limitation may even affect cells in the centre of a singe colony (Paerl 1983).

(2) Photooxidation may reduce the photosynthetic rate required to sustain a buoyancy increase (Abeliovich and Shilo 1972; and see Kromkamp, this volume).

(3) Cyanobacteria that have previously circulated deep in the water column may become so highly buoyant with gas vesicles that they are unable, in the course of a day, to accumulate enough carbohydrate ballast to sink. *Anabaena lemmermanni* in Windermere was found to become trapped at the water surface in this way; it could not escape by the alternative mechanism of destroying its gas vesicles because in this species they were too strong to be collapsed by turgor pressure (Walsby et al. 1991).

(4) In very thick layers of surface blooms such as accumulate towards leeward shores, the cyanobacteria at the surface may become buoyed up by others below them, which in turn remain buoyant because of the steep attenuation of light in the surface scum (Zohary and Robarts 1989; Robarts and Zohary 1984).

Although waterblooms may be continuously present for days or weeks on some lakes it does not follow that it is the same colonies that persist at the water surface. Kromkamp and Walsby (1990) showed by computer modelling that the appearance of a continuous bloom may be given by an overlapping succession of colonies, each of which spends only a few hours at the water surface. Direct evidence is now needed on this point. It might be obtained by pulse labelling colonies at the surface with $^{14}CO_2$ and then tracking their subsequent changes in vertical distribution.

3.2. BUOYANCY IN *TRICHODESMIUM*

3.2.1. *Floating and sinking velocities.* The floating velocities of *Trichodesmium* have been measured by introducing them at the bottom of a 10 ml graduated measuring cylinder and timing their rise over a distance of 42 mm. Velocities of between 0.1 and 3.0 mm s^{-1} were recorded for buoyant colonies found floating at the ocean surface. The fastest rates were found for the colonies of *T. erythraeum*, some of which measured 2 mm across. After their gas vesicles had been collapsed by application of pressure many of the colonies sank at a comparable velocity, as expected if the sinking velocity is mainly determined by size (Walsby 1978).

There have been no direct determinations of the density of the colonies but an approximate range can be estimated from the Stokes equation (2). Taking u = 0.5 mm s^{-1}, r = 1 mm, g = 9.8 m s^{-2}, and h = 0.001 kg m^{-1} s^{-1}, then the excess density would be 1.8 kg m^{-3}. The excess density of individual cyanobacterial cells or filaments (with gas vesicles collapsed) is usually of the order of 50 kg m^{-3} (Utkilen et al., 1985, Thomas and Walsby 1985); this suggests that only 3.7% of the colony volume is occupied by filaments. A similarly low proportion occurs in colonies that are occasionally formed by *Oscillatoria agardhii*

(Walsby et al., 1983b; Kromkamp and Walsby 1990). Direct determination of colony density by the Percoll density gradient methods of Oliver, Kinnear and Ganf (1981) are now needed.

The floating velocities of *Trichodesmium* are among the fastest recorded by planktonic cyanobacteria. Rapid rates of rise must be needed to return the colonies to the surface layers of the ocean after deep mixing has occurred. Even these high rates will have been underestimated because they were made in a vessel whose diameter was not large compared with the diameter of the colonies (See McNown and Malaika, 1950).

3.2.2. *Buoyancy regulation.* The observation that *Trichodesmium* populations formed maxima 15 or 20 m below the ocean surface (Carpenter and McCarthy 1975; Carpenter and Price 1977) suggested that these cyanobacteria might be regulating their buoyancy. In keeping with this idea a mixture of floating and sinking colonies were found in net hauls from depths of 10 m and more floating colonies were found in the morning than later in the day (Walsby 1978). No loss of buoyancy was found in colonies that were incubated in full sunlight for several hours, however, perhaps because the cyanobacteria became photoinhibited (Li et al. 1980).

In more detailed experiments Villareal and Carpenter (1990) obtained direct evidence for buoyancy regulation. By leaving colonies, collected from the ocean, in a settling chamber for two hours, they separated them into three buoyancy classes, floating, neutrally buoyant and sinking. They then incubated these colonies for two hours either at a photon irradiance of 127 mol m^{-2} s^{-1} (about 6% of full sunlight) or in darkness. About 60% of the floating colonies became neutrally buoyant in the light but they remained buoyant in the dark. Over 70% of the neutrally buoyant colonies sank after the light treatment, whereas after the dark treatment a similar proportion floated. All of the colonies that initially sank remained sinking after the light treatment while after 2 hours darkness there were equal proportions of floating, neutrally buoyant and sinking colonies. Villareal and Carpenter (1990) also demonstrated that in samples collected from the ocean at different times of day the proportion of colonies that were buoyant decreased with time after dawn and increased after dusk (see Fig. 2). This suggested that the colonies in the sea were changing their buoyancy in response to changes in the natural light field.

The mechanism of buoyancy regulation in *Trichodesmium* is not yet known. The gas vesicles are clearly much too strong for the turgor-collapse mechanism to operate (Walsby 1978) and it is likely that the accumulation of carbohydrate

ballast causes buoyancy loss in the light, as occurs in many of the freshwater cyanobacteria.

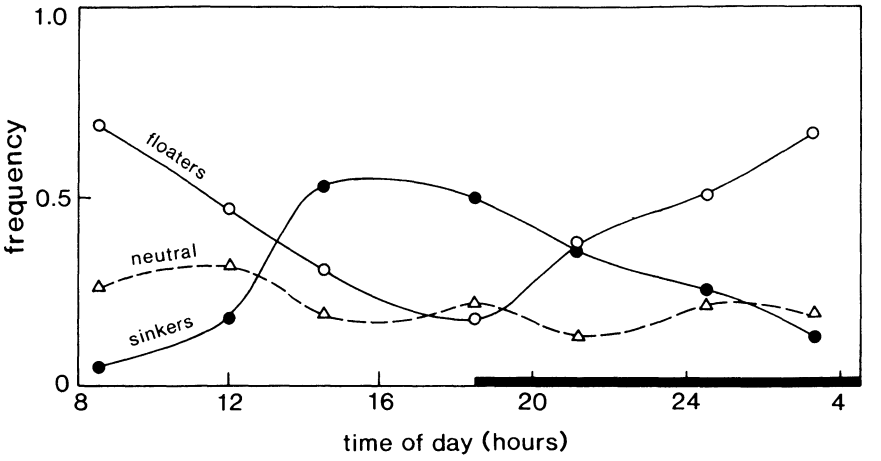

Fig. 2. The frequency of *Trichodesmium* colonies, collected at different times of day from the ocean, that were positively, neutrally or negatively buoyant. The bar indicates hours of darkness. Redrawn from data of Villareal and Carpenter (1990).

3.2.3. *Buoyancy regulation and vertical migration.* It is clear from the results of (Villareal and Carpenter 1990) that the colonies of *Trichodesmium* will perform vertical migrations. From information on the buoyancy changes that occur and the speed at which they do so predictions can be made of the likely amplitude and periodicity of their peregrinations by using the computer model. Kromkamp (this volume) makes such predictions which should be of value in planning sampling programmes to collect evidence on this.

3.2.4. *Waterblooms and surface slicks.* There is no evidence that *Trichodesmium* accumulates at the surface by growing there. Its growth in the intense irradiance at the surface is inhibited by photooxidation (Li et al. 1980), and the organism can rarely be cultured from samples collected at the surface. It is more likely that the surface accumulations of *Trichodesmium* form from populations that have grown in subsurface layers and then floated up, as occurs in cyanobacterial blooms in freshwater lakes (see 3.1.4.). This is supported by reports that surface blooms of *Trichodesmium* develop under conditions of calm and that the blooms are largely confined to the top few centimetres

of water (Devassy et al. 1978; and see Fogg 1981).

We do not know how long individual colonies spend at the water surface but it seems likely, from the poor metabolic rates of surface colonies, that many of them become trapped there by their buoyancy, like the *Anabaena lemmermannii* in Windermere (see 3.1.4.). Their gas vesicles are so strong that they do not have the possibility of collapsing them by turgor pressure. It is not known if they have other methods of destabilising their gas vesicles.

4. Epilogue

We conclude where Banks began, with his report of sea sawdust floating upon the water. Sea sawdust exactly describes the appearance of *Trichodesmium* colonies to the unaided eye : they float, they have the colour of wood chips, and they are particles of only a millimetre across. Now we know the cause of their buoyancy. Their reddish coloration, provided by phycoerythrin for photosynthesis in green light and by carotenoids for photoprotection, is also understood. I will conclude with some speculations on colony size.

4.1. COLONY SIZE

Cyanobacteria have two strategies for suspending themselves in the oceans. The picoplanktonic forms, which are everywhere, have infinitesimal sinking speeds by virtue of their small size. Species of *Trichodesmium*, which are restricted mainly to the tropics, float by having large colonies buoyant with gas vesicles.

In the cooler temperate oceans the conditions may be so seldom calm enough to permit colonies to float back to the surface, that their vertical distribution would be no different from that of the picoplankton. Both would be circulated through the surface mixed layer. In a competition between the two, the picoplanktonic cyanobacteria would be expected to win because of their greater efficiency in absorbing nutrients (Munk and Riley 1952) and light (Raven 1988). Moreover, they would not have to bear the additional costs of making gas vesicles. In *Anabaena flos-aquae* about 8% of the cell protein is devoted to the construction of gas vesicles in buoyant cells (data of Walsby and Armstrong 1979 and Oliver and Walsby, 1984). For the narrower gas vesicles of *Trichodesmium thiebautii* about 15% of the cell protein would be required to provide the same degree of buoyancy.

In tropical waters the temperature gradients are steeper and the vertical shear velocities will more often fall below the upward floating velocities of the colonies. Whenever this occurs the buoyant colonies will be

distributed closer to the surface than the picoplankton and will win the competition for light (Humpries and Lyne 1988).

What, then, limits the size of the colonies? Is it the limitations set by rates of nutrient uptake and light absorption discussed above? Discounting the aggregations of colonies that may form in surface waterblooms the colonies of *Trichodesmium* are among the largest and they may be pushing the limits. Perhaps these limits may be somewhat higher for filamentous cyanobacteria (like *Trichodesmium* and *Aphanizomenon*) than unicellular ones (like *Microcystis* and *Coelosphaerium*) because, while the positions of the unicells may be fixed, the filaments may move over one another. They may therefore take it in turns to sample the nutrients at the colony surface, or seek shade in the colony centres. Moreover, the cells of filaments can cooperate in transporting nutrients along their lengths.

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MARINE NITROGEN-FIXING DIATOM-CYANOBACTERIA SYMBIOSES

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ABSTRACT. This review summarizes growth and nitrogen-fixation data on diatom-cyanobacteria symbioses. Symbiotic associations between diatoms and filamentous cyanobacteria are frequently noted in tropical and subtropical waters, and direct evidence indicates that at least two associations are diazotrophic. Abundance data is limited, but *Rhizosolenia-Richelia* blooms are recorded from the central Pacific gyre at up to 10^4 cells L^{-1}. *In-situ* growth rates of these associations are not known; however, laboratory data on the *Rhizosolenia-Richelia* symbiosis suggests the host-symbiont association can reproduce at approximately 0.8-1.0 div day^{-1}. Growth kinetics in nitrogen-poor medium suggest that transfer of fixed nitrogen from host to symbiont occurs and that this process can support the host's growth under N-limiting conditions. Additional symbioses, particularly in *Hemiaulus*, may be more widespread than previously realized due to the difficulty in identifying the symbiont in standard light microscopy. Few field measurements of N_2-fixation are available for these associations. *Rhizosolenia-Richelia* appears to be locally important in the central Pacific gyre. *Hemiaulus* spp. are a common and abundant diatom in oligotrophic seas, and if 80-100% of the cells are symbiotic as reported, then they represent an unquantified, and potentially substantial, source of nitrogen-fixation.

1. Introduction

1.1. TYPES OF DIAZOTROPHIC DIATOM-PROKAYROTE SYMBIOSES

Several types of diatom-prokaryote symbioses are common in marine waters. The first, and most frequently reported, is the association of filamentous, heterocystous cyanobacteria with the diatom genera *Rhizosolenia, Hemiaulus, Bacteriastrum,* and *Chaetoceros* (Karsten, 1905, 1907; Sournia 1970). Nitrogen fixation has been clearly demonstrated by the symbiont of *Rhizosolenia* and *Hemiaulus*. The second association is of unicellular, coccoid cyanobacteria with the genus *Streptotheca/Neostreptotheca*. These associations have not been conclusively documented to be diazotrophic, although similar associations in freshwater systems between coccoid cyanobacteria and the diatom *Rhopalodia* are known to fix nitrogen (Floener and Bothe, 1980). The third type of symbiosis occurs in *Rhizosolenia*, where intracellular bacteria

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capable of acetylene reduction are reported in two species (Martinez et al., 1983). To date, this association has only been noted from oceanic diatom mats, and will not be discussed in this review (see Villareal and Carpenter, 1989). The remainder of this review will discuss the biology and nitrogen-fixation capabilities of diatom-cyanobacteria associations.

1.2 TAXONOMIC NOTES

At least three filamentous, heterocystous cyanobacteria have been reported in diatoms. The most commonly noted, and most easily observed, is *Richelia intracellularis* Schmidt (Ostenfeld and Schmidt, 1901). This species is reported as both an endo- and exo- symbiont, occurring within or on cells of *Rhizosolenia* and *Hemiaulus*, *Bacteriastrum*, and *Chaetoceros*. As an endobiont in *Rhizosolenia*, the symbiont occurs at the extremities of the cell, and is visible even under low magnification (Fig. 1).

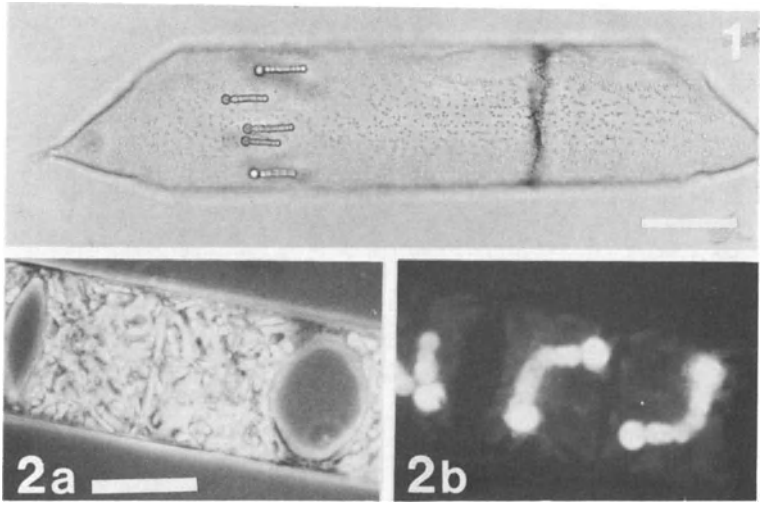

Fig. 1. Multiple *Richelia* trichomes contained in a large cell of *Rh. clevei*. Scale=100 μm. Fig. 2.Symbiont -containing cells of *H. membranaceus* Cl. Fig. 2a. Phase contrast. Note the symbionts are not visible. Fig. 2b. Epi-fluorescent illumination showing the symbionts. Fig. 2 photographs from Villareal (1991) and were provided by D. A. Caron. Scale bar=10 μm.

An additional taxon, *Calothrix rhizosoleniae* Lemmermann (Lemmermann, 1905) has been described, but it is infrequently reported. The two species differ primarily in the diameter of the basal heterocyst, the length of the trichome, and the number of cells in the trichome (Table 1). *C. rhizosoleniae* is also reported to have a sheath, and that the trichome tapers away from the heterocyst (Lemmermann, 1905, Sournia, 1970). The similarity of *R. intracellularis* and C. *rhizosoleniae* suggests taxonomic confusion exists (Sundström, 1984), and a clear delineation of the two species is required to evaluate fully any potential host specificity. Norris (1961) noted that the epiphyte (termed *Richelia*) was a different color (pinkish-grey) than the endophyte (light blue green) and suggested phycobilin differences (phycoerythrin versus phycocyanin) occurred as a result of the differing habitat (Norris, 1967). Drouet (1973) considered *Richelia* to be synonomous with *Calothrix crustacea* Thuret, but his

description suggests he never saw living intracellular symbionts, and his nomenclature will not be used. Taylor (1983) has cited anecdotal accounts of a potentially diazotrophic

Table 1. Comparison of reported Richelia intracellularis and Calothrix rhizosoleniae dimensions. Measurements are in µm.

Species	Heterocyst diameter	Cell diameter	Cells/ trichome	Trichome length
Richelia	8-13.5	5	-	40-80[1]
	-	-	6-14	-[2]
	9-11.2	5-9	7-20	50-105[3]
Calothrix	3-6	1.5-3.5	4-8	10-18[4]

[1]Sundström 1984, [2]Taylor 1982, [3]Ostenfeld and Schmidt 1901, [4]Lemmermann 1905

Anabaena symbiont in *Coscinodiscus*. This may be the same as an observed association between *Roperia tessellata* (Roper) Grun. and an unidentified *Nostoc*-like species observed by G. R. Boalch (per. comm.), but no additional observations are available. Wood (1965) reports the occurrence of a *Richelia*-like symbiont on a benthic *Pleurosigma*.. For the purposes of this paper, all filamentous, heterocystous cyanobacteria found in *Hemiaulus* and *Rhizosolenia* will be refered to as *Richelia*.

A significant problem exists in identifying many diatom symbionts, particularly in *Hemiaulus*. The cytoplasmic mass of the host diatom can obscure the symbiont and render it virtually invisible in standard light microscopy. The use of epi-fluororescence microscopy has revealed that significant numbers of *Hemiaulus* cells contain symbionts (Fig. 2) that are not seen under bright field microscopy (Heinbokel, 1986; Villareal, in press). Additional symbioses may exist that have not been identified for similiar reasons.

2.0. ***Rhizosolenia-Richelia***

2.1 *RICHELIA* HOSTS

Up to 12 species of *Rhizosolenia* have been reported to contain the endosymbiont *Richelia intracellularis* (*Rh. acuminata* (H. Perag.) Gran, *Rh. bergonii* H .Perag., *Rh. castracanei* H. Perag., *Rh. clevei* H. Perag., *Rh. cylindrus* Cl., *Rh. firma* Karst., *Rh. hebetata* Bail, *Rh. imbricata* Bright., and *Rh. styliformis* Brightw.) by various authors (Hustedt, 1930; Sournia, 1970; Taylor, 1982). Several additional hosts (*Rh. alata* Brightw., *Rh. setigera* Brightw., *Rh. similis* Karst. and *Rh. temperei* Pera.) are questionable (Sournia, 1970). Sundström (1984) suggests that many of these reports misidentified the host, and most, if not all, of the hosts were actually *Rh. clevei* varieties. The host *Rh. clevei* size ranges from 7-250 micrometers diameter and contains 1->32 symbionts (Sundström, 1984; Villareal, 1990). The small end of the range apparently represents the minimum diameter that can accommodate the

symbiont, since asymbiotic *Rh. clevei* cultures will continue to grow in the absence of their symbiont if nitrogen is supplied (Villareal, unpub. obs.). It is not known what factors other than host size limits the number of symbionts per cell. There presumably is a relationship between host-symbiont biomass as has been shown in other cyanobacteria-eukaryotic plant symbioses (Stewart et al., 1983).

2.2 SYMBIONT LOCATION AND DIVISION CYCLES

The symbiont is usually described as contained in the host cytoplasm. However, Sweeney (cited in Fogg,1982) observed that the host cytoplasmic mass in preserved material easily separated from the *Richelia* and suggested that the symbiont was extracytoplasmic. Villareal (1990) noted similar host-symbiont separation in both Lugol's iodine and formalin- preserved cells, and was able to stain deteriorating symbionts in living hosts with stains that do not pass intact plasmalemmas. Based on the classification of Smith and Douglas (1987), the symbiont is therefore considered an extracellular endosymbiont contained in the periplasmic space between the cytoplasm and the cell wall. As noted below, the location of the symbiont has implications for the possible excretion of recently fixed nitrogen into the surrounding medium.

Richelia is usually located at the apex of the host *Rhizosolenia* cell, in or near the valve. The basal heterocyst is always at the end nearest the valve. Transmission occurs by elongation of the trichome, enlargement of a new terminal cell, and separation and migration of the trichome to the opposite valve (Taylor, 1982). Trichome migration occurs at approximately 5 $\mu m\ sec^{-1}$ and is independent of the host cytoplasmic streaming (Villareal, 1989). *Richelia* trichome division occurs more or less continuously during the diel cycle, and no evidence of photopheriod-phased separation was noted. The host *Rhizosolenia* was observed to undergo phased cell division early in the morning. As a consequence of these uncoupled division cycles, asymbiotic *Rhizosolenia* were a common occurrence in the cultures (Villareal, 1989).

2.3 ABUNDANCE AND DISTRIBUTION

Richelia is primarily found in warm tropical and subtropical waters, with a latitudinal range of approximately 38° 09'N (Venrick, 1974) and 36° 30' S (Sundström, 1984). The host was observed north of this region in Transition zone waters by Venrick (1974). Marumo and Asaoka (1974) also noted the symbiont was found only the gyre, although the host was noted in both equatorial and in subarctic waters. However, *Richelia* has been observed in the equatorial current during periods of calm when vertical stratification is strongest (E. Bauerfeind, per.comm.). It is rarely noted in coastal waters (Marshall, 1981) unless it is associated with an intrusion of offshore waters, such as the high salinity wedge occurring at the mouth of the Chesapeake Bay (H.G. Marshall, per. comm.). Host distributional data is difficult to interpret in light of Sundstrom's taxonomic revision of the genus *Rhizosolenia*, and his report that *Rh. clevei* is the primarily host of *Richelia*. Sundstrom's observations indicate that *Rh. clevei* is always found containing the symbiont, and that the true *Richelia* was never seen in other *Rhizosolenia* species. He further asserts that many reports of *Rhizosolenia* species other than *Rh. clevei* containing *Richelia* are misidentifications. In cases such as *Rh. cylindrus* (a distinctive species unlike *Rh. clevei*), the symbiont may well represent a different taxon such as *C. rhizosoleniae* . The inferred obligate nature of the *Rh. clevei-Richelia* symbiosis may only occur in the field since Villareal (1990) has noted that even under N-

deplete culture conditions, asymbiotic host cells occur. These asymbiotic cells grow easily in standard diatom culture medium, and do not appear to have unusual nutritional requirements. In addition, several reports have noted blooms of *Rhizosolenia-Richelia* with only 94-98+% of the *Rhizosolenia* with symbionts (Venrick, 1974; Taylor 1982). Villareal (1990) suggested the two disparate observations may be resolved by a high selection for symbiotic *Rhizosolenia clevei* in nitrogen-poor seas, while bloom conditions (presumably less nutrient limited) may permit asymbiotic *Rhizosolenia* to persist for some time.

Limited data is available on the actual abundance of *Richelia*. Although frequently noted, it is rarely enumerated. Marshall (1982) noted average occurrences of 3.1 trichomes per liter off the eastern U. S. coast. Saino (1977) reported a maximum of approximately 20 *Richelia* trichomes L^{-1}. Venrick (1974) provides the most complete coverage (Fig. 3), with seasonal data for the central North Pacific gyre.

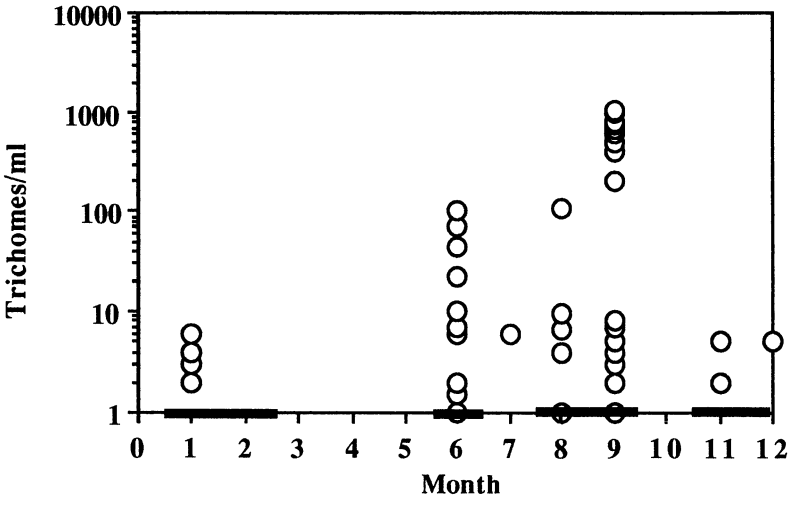

Fig. 3. Seasonal abundance of *Richelia* in in the central North Pacific gyre from 1964-1973. Points represent the maximum abundance of *Richelia* at a single station. Horizontal dark bars indicate the presence of samples with no *Richelia* (after Venrick, 1974).

Abundance spans approximately 3 orders of magnitude with winter abundance of 10 cells L^{-1} or less up to greater than 10, 000 cells L^{-1} in summer blooms. The summer blooms appear to be an annual occurrence, but are apparently localized, independent events representing a bloom, decline and physiological collapse of unrelated populations (Venrick, 1974). There was no clear relationship of the blooms to ambient nutrients, and factors inducing blooms are unknown. Atmospheric inputs may be important in supplying sporadic inputs of limiting trace metals such as iron (Duce, 1986; Rueter, 1988), but the problem remains unresolved.

2.4 FIELD STUDIES: NITROGEN-FIXATION AND COMMUNITY RESPONSE

Mague et al. (1974) provided the first compelling evidence of nitrogen-fixation by *Richelia* using net tows and filter-concentrated material in the central North Pacific gyre. The host populations in this study appeared moribund as evidenced by the lack of cytoplasmic streaming (Mague et al., 1974), clumping of host *Rhizosolenia* cells (Venrick, 1974), and the lack of autoradiographic ^{14}C labelling of the host *Rhizosolenia* (Weare, et al., 1974). Acetylene reduction rates were depressed 70-80% by filtering net-collected material onto glass fiber filters (Mague et al. 1977; Mague et al. 1974), hence rates reported in these studies must be treated as minimum. Maximum acetylene reduction rates (565 pmol $C_2H_4^{-1}$ hr^{-1}) occurred at 25-30 m, was well above the deep chlorophyll maximum (Venrick 1974), and 10-15 meters above the *Richelia* cell maximum in samples collected 15 km away. Trichome specific rates of acetylene reduction were calculated from their data to be 0.95-1.9 X 10^{-12} M C_2H_4 trichome hr^{-1} (Villareal 1990). Using net collected material, acetylene reduction was shown to be light dependant, with saturation occurring at about 5% of incident light or 105 μE m^{-2} sec^{-1} (estimated). Darkened material ceased ethylene evolution after 11 minutes. Re-illuminated material started evolving ethylene after 7 minutes and reached maximum rates within 11 minutes. An apparent stimulation by phosphate additions was noted by Mague et al. (1974) but not found by Mague et al. (1977). Differences in phosphorus sufficiency were suggested as an explanation.

Mague et al. (1974) calculated approximately 800 μg N m^{-2} day^{-1} or 4% of the total phytoplankton N needs were supplied by the *Richelia* during bloom conditions in 1972. Venrick (1974) calculated the potential input by *Richelia* during the 1969 bloom to be 6.2-12.5 milligrams N m^{-2} day^{-1} or approximately 12-25 % of the total production and 30-60% of the excess production above non-bloom conditions.The observed 70-80% depression of rates due to filter collection (Mague et al. 1977) suggests that actual contribution was significantly higher. When corrected for these handling artifacts, Mague et al.'s (1974) field rates (approximately 3.8-7.6 X 10^{-12} M C_2H_4 trichome hr^{-1}) are comparable to light saturated laboratory rates of *Richelia* acetylene reduction (9.5 X 10^{-12} mol C_2H_4 trichome hr^{-1}, Villareal 1990).

Richelia blooms were accompanied by elevated ambient chlorophyll concentration in the mixed layer, and, in 1969, by primary productivity increases over non-bloom years (Venrick, 1974). Venrick estimated the bloom of 1969 covered in excess of 3,500 km^2. Total phytoplankton biomass was elevated in the upper 60 m and the deep chlorophyll maximum appeared to be weaker than usual when *Richelia* blooms were present. Diatoms, in particular, responded to bloom conditions with both *Rhizosolenia* and *Hemiaulus* reaching 10^4 cells L^{-1}. As noted below, *Hemiaulus* may have also contained symbionts. Bloom termination was accompanied by increasing numbers of moribund *Rhizosolenia* and an increased tendency to clump into raft-like aggregations. The ultimate fate of these blooms is not known, but the aggregation behavior suggests that sedimentation is a large potential sink.

2.5 LABORATORY STUDIES: GROWTH AND N_2-FIXATION

Laboratory investigations of *Rhizosolenia-Richelia* have indicated that the host *Richelia* can supply the host's N needs under N-deplete conditions (Villareal, 1990). Asymbiotic *Rhizosolenia* persisted in N-deplete conditions, and accounted for approximately 35 % of the total *Rhizosolenia* at stationary phase. A comparison of symbiotic and asymbiotic growth

rates under N-deplete and N-replete conditions found that symbiotic *Rhizosolenia* grew at the same rate in both media, while N-deplete asymbiotic *Rhizosolenia* had their growth rate reduced to 60% of rates with symbionts (Table 2).

Richelia growth rate was higher in N-replete medium (0.80 versus 0.67 trichome div. day^{-1}). Under N-replete conditions, the host had a slightly higher growth rate than the symbiont. Although the two growth rates were not statistically different, under N-replete conditions asymbiotic *Rhizosolenia* cells constituted a much higher percentage of total *Rhizosolenia* than they did under N-deplete conditions (Fig. 3).

Table 2. Growth rates (div. day^{-1}) of the host Rhizosolenia and the symbiont Richelia in N-deplete and N-replete media (from Villareal, 1990)

Medium	Total Host	Symbiotic Host	Asymbiotic Host	Richelia
N-replete	0.72	0.81	0.71	0.80
N-deplete	0.44	0.77	0.35	0.67

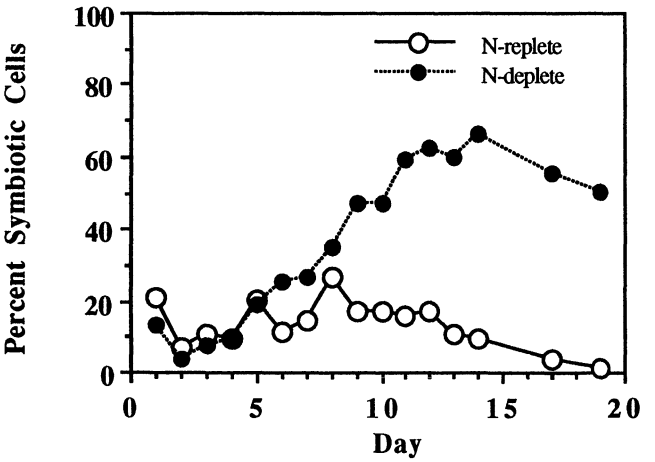

Fig. 3. Percent of *Richelia*-containing *Rhizosolenia* and non-symbiotic *Rhizosolenia* in nitrogen replete (40 µm NO_3^{-2}) and nitrogen-deplete (<1.0 µm NO_3^{-2}) media (from Villareal, 1990).

Citrate additions resulted in bacterial growth and no asymbiotic *Rhizosolenia* growth, although symbiotic *Rhizosolenia* grew at the expected rates. Villareal (1990) suggested that this data could be interpreted as fixed nitrogen excretion by the symbiont *Richelia* which was available for asymbiotic *Rhizosolenia* utilization in control cultures, but not in citrate-enhanced cultures with intense bacterial nitrogen utilization. This explanation requires the symbiont to be able to directly excrete nitrogen into the medium, a requirement supported by the observations of the extracytoplasmic location of the symbiont.

Host-symbiont growth followed Jassby-Platt light saturation kinetics (Table 3). Maximum host growth rate (1.04 div day-1) under N-replete conditions was not significantly different than the symbiont (0.80 trichome div. day^{-1}), although as noted above, the asymbiotic Rhizosolenia dominated the culture under high light conditions. No significant difference existed between the initial slope of the Jassby-Platt fit for host and symbiont, but under N-replete conditions the host I_k was 2-4 times higher than the symbiont's I_k. Compensation intensities had significant errors associated with them, but were in the range of 4±10 μE m^{-2} sec^{-1}.

Nitrogen fixation saturated at 100-125 μE m^{-2} sec^{-1}. Maximum acetylene reduction rates were 9.5 $X10^{-12}$ mol ethylene trichome hr^{-1}. Based on a nitrogen budget, the estimated acetylene reduction: nitrogen fixation ratio was ≤11. Dark fixation was noted in these late photoperiod experiments, suggesting the actual ratio would be less.

There is currently an inadequate data base to calculate global N_2 fixation by *Rhizosolenia-Richelia*, making any attempt at global rates inaccurate and possibly misleading. Based on the available abundance information, it does appear that the global contribution of *Rhizosolenia-Richelia* is less than *Trichodesmium*, and that it is important primarily in localized intense blooms.

Table 3. Host-symbiont growth parameters for the Rhizosolenia-Richelia symbiosis (from Villareal, 1990).

Species	Media	gamma(1)	μ_{max}(2)	I_k(3)
Rhizosolenia	N-deplete	.011	0.92	83
Richelia	N-deplete	.015	0.87	63
Rhizosolenia	N-replete	.010	1.04	176
Richelia	N-replete	.017	0.80	47

(1) div day^{-1} (μE m^{-2} sec^{-1})-1 (2) div day^{-1} (3) μE m^{-2} sec^{-1}

3.0. *Hemiaulus* symbionts

Four species of *Hemiaulus* (*H. indica* Karst., *H. hauckii* Grun., *H. membranaceus* Cl., *H. sinensis* Grev.) are reported to contain *Richelia* symbionts (Fig. 2, see taxonomic discussion above, Sundström, 1984; Villareal, in press). Kimor et al. (1978) noted up to 62% of the *Hemiaulus* off California contained the symbiont, but offshore samples contained only 16.1-

18.2 % symbiotic cells. Recently, Heinbokel (1986) noted that epifluorescent illumination revealed approximately 80% of the *Hemiaulus* in net tows near Hawaii contained symbionts, while standard light microscopy revealed no symbionts. He further suggested the abundance and importance of the symbiont may be greater than realized due to the cryptic nature of the symbiont. Villareal (in press) documented acetylene reduction under aerobic conditions in the southwest Atlantic ocean from hand-picked cells of *Hemiaulus*, and reported an average rate of 3.6 X 10^{-13} moles ethylene $cell^{-1}$ hr^{-1}. These rates were 2.2-5.3 times lower than field rates of ethylene evolution calculated for Mague et al.'s (1974) Pacific *Richelia* bloom. He further noted 100% of the living *Hemiaulus* contained 1-2 symbionts.

Hemiaulus abundance reaches 140,000+ cells L^{-1} in coastal seas (Saunders and Glen, 1969) and 25,000 cells L^{-1} in oligotrophic seas (Venrick, 1974). Villareal (in press) calculated that 18-45 times as much acetylene reduction was occurring by *Hemiaulus* symbionts as by the *Rhizosolenia* symbionts in the southwestern Atlantic Ocean. The importance of this phenomenon is impossible to determine at present because of the uncertain presence of symbionts in other *Hemiaulus* populations. Assuming one symbiont per *Hemiaulus*, Villareal (in press) calculated that the observed *Hemiaulus* abundance (~100 cells $L^{-1)}$ may contribute 15% of *Trichodesmium* N_2 fixation in the central North Pacific gyre. Should the reported blooms of *Hemiaulus* in this region contain symbionts, the contribution would be proportionally higher.

4.0 Coccoid cyanobacteria

Norris (1967) reported the occurrence of unicellular cyanobacteria (*Synechococcous carcerarius* Norris) in *Strepotheca indica* Karst. The cyanobacteria ranged from 8-10 X 3-5 micrometers in size. Hallegraeff (1984) reported a similar association from Australian waters in *Neostreptotheca subindica (*. The association appears to be similar to a coccoid cyanobacteria in the freshwater *Rhopalodia gibba* (Ehr.) O. Mull. as well as in *Rhopalodia gibberula*, *Epithemia sorex* (Kütz.) Grun., E. *turgida* (Ehr.) Kütz. *E. zebra* (Kütz.) Grun.and *Denticulata vanheurckii* Brun. (Geitler, 1977) *Rhopalodia gibba contains* 2-5 ellipsoidal to coccoid cyanobacteria cells that reduce acetylene aerobically at rates of 10 nmol C_2H_4 mg $protein^{-1}$ hr (Floener and Bothe, 1980). Symbiont abundance in *Rhopalodia gibba* was dependent on nitrogen source and concentration (DeYoe, 1991). The possibility that similar dynamics occur in *Strepotheca/Neostreptotheca* cannot be discounted.

5.0 Discussion

The available evidence suggests that some diazotrophic symbioses are capable of rapid growth and form localized blooms, particularly in the oligotrophic central Pacific gyre. Laboratory results indicate that the *Rhizosolenia* host can benefit directly from symbiont nitrogen fixation, thus providing a strong selective pressure for maintaining the symbiosis. Such a nitrogen transfer would greatly aid the survival of the host in nitrogen-poor seas (Guillard and Kilham, 1977). The symbiosis does not appear to be obligate in *Rhizosolenia*, although there is inadequate field data to determine whether such asymbiotic cells are a laboratory artifact. *Hemiaulus* symbioses are more abundant than *Rhizosolenia* symbioses where direct comparisons have been made, and at least in the Caribbean, were found to fix more nitrogen.

The cryptic nature of the *Hemiaulus* symbiont suggests it has been overlooked previously, and it may be more important in water column nitrogen fixation than previously believed. Other symbiotic associations in diatoms are known to occur, but are poorly characterized.

The central role of N_2 fixation in the symbioses suggests that factors limiting nitrogen fixation such as iron or phosphorus (Rueter and Petersen, 1987) should also be important in the dynamics of these associations. In addition, the host diatom has an obligate requirement for silicate (Paasche, 1980) that is in addition to N_2 fixation requirements of the symbiont. This latter requirement may create a fundamental difference in the bloom dynamics of *Trichodesmium* and diazotrophic diatom symbioses. Mague et al. (1974, 1977) noted *Trichodesmium* and *Rhizosolenia-Richelia* blooms separately, but did not report their co-occurence. It would be useful to know if blooms of these two diazotrophs co-occur or are generally mutually exclusive.

Trichodesmium is capable of positive buoyancy and rapid buoyancy reversals that probably permit depth-keeping or vertical migration (Walsby, 1978; Villareal and Carpenter, 1990; Kromkamp this volume). Such characteristics are undoubtedly related to the surface blooms that occur during calm weather (Carpenter, 1983). While the largest *Rhizosolenia* are capable of positive buoyancy (Villareal and Carpenter, 1989), at this time it is unclear whether *Richelia*-containing *Rhizosolenia* can also ascend. It is unknown whether *Richelia* possesses gas vacuoles. The observed diatom symbioses blooms in the Pacific typically occur 10's of meters deep in the euphotic zone, not at the surface as in *Trichodesmium*. It is possible that sinking and sedimentation, in addition to silicate requirements, are also important factors in selecting for a *Trichodesmium* versus a diatom symbiosis bloom. Grazing should also be considered, since the presence of toxins in *Trichodesmium* (Hawser et al. 1991) may inhibit grazing. *Richelia* symbioses have not been examined for toxins.

The lack of abundance and rate data for symbiotic diatom associations limits our current understandings. Future research needs are identification and quantification of symbioses using fluorescence microscopy, and determination of the nitrogen fixation rates associated with symbioses. Comparative studies of diatom symbioses and *Trichodesmium* could also provide useful insights into similar factors limiting nitrogen fixation in both symbiotic and free-living cyanobacteria in the sea, as well as their unique requirements. Host-symbiont regulation in diatom-cyanobacteria symbioses are probably not substantially different from symbiotic cyanobacteria in terrestrial and aquatic habitats (Peters, 1978; Stewart et al., 1983), thus the extensive literature on these associations should provide useful models for regulation and transfer mechanisms.

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DISCOLOURED WATERS IN THE MELANESIAN ARCHIPELAGO (NEW CALEDONIA AND VANUATU). THE VALUE OF THE NIMBUS-7 COASTAL ZONE COLOUR SCANNER OBSERVATIONS.

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ABSTRACT. Final conclusions are given to the study of discoloured waters as they can be observed with the NIMBUS-7 Coastal Zone Colour Scanner (CZCS) in the oligotrophic waters of the southwestern tropical Pacific, in the Melanesian archipelago, around New Caledonia, Vanuatu (and Tonga). An attempt was made to define, in the four CZCS channels, the reflectance properties of these blooms attributed to *Trichodesmium*, a nitrogen-fixing cyanobacterium commonly found in that region. The capacity and limitations of the CZCS sensor used in the past as well as the SPOT imager are discussed in this respect. The usefulness of past and future ocean colour satellites in identifying zones of high chlorophyll concentration and bright linear patterns is demonstrated.

1. Introduction

The goal of the study was to determine how ocean colour imagery could help to characterize the *Trichodesmium* blooms commonly found in the Melanesian archipelago, around New Caledonia, Vanuatu (and Tonga) in the southwestern tropical Pacific. In a first paper (Dupouy *et al.*, 1988), a CZCS image of a particularly bright and extensive area of discoloured water, presumably caused by *Oscillatoria (= Trichodesmium)*, was described in detail. Using *in situ* data from Capone and Carpenter (1982), we were able to estimate its nitrogen fixation capacity at about one ton.

An examination of the whole CZCS series for the region (Dupouy, 1990a,b) gave new insight into the distribution of surface chlorophyll in the Melanesian archipelago. Conclusions were that discoloured waters occur primarily in summer (December to March) and that surface chlorophyll-rich zones usually extend far eastward from New Caledonia, (over 500 km) on the convergence zones of currents.

The purpose of this study was to determine if these observations could be generalized to provide a detecting method of the *Trichodesmium* blooms. At first, a review was made of the occurrence of *Trichodesmium* in the western Pacific, and of its repetitivity in the Caledonian archipelago (Loyalty Islands) based on *in situ* ORSTOM data. Secondly, the CZCS four band reflectances were examined for blooms at different dates. The SPOT imagery available over the Caledonian lagoon was used in order to illustrate the spatial organization of the bloom on a much finer scale.

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2. Occurrence of *Trichodesmium* in the western Pacific and in the Melanesian archipelago

According to Soumia (1968, 1970), the marine cyanobacterium (blue-green alga) genus *Trichodesmium* can be assumed to be the genus *Oscillatoria (= Trichodesmium)* Vaucher ex Gomont, in the order Hormogonales.

A review of the occurrence of *Trichodesmium* in the Pacific has been made by Carpenter (1983). In the northern hemisphere, between 1892 and 1950, several naturalists observed blooms during their exploratory cruises, in the Java Sea, near Borneo, in Indonesian regional seas, the East China Sea, in waters around Japan and in the Kuroshio current (as referenced by Bowman and Lancaster (1965) and Asaoka and Marumo (1987)). Similarly, in the southern ocean, the first surveys confirming the occurrence of *Trichodesmium* blooms were made near the Guinea coast, in the Arafura Sea and on the Great Barrier Reef. Recent studies have been made on the GBR region (Revelante and Gilmartin, 1982, Hallegraef and Reid, 1986). The western Australian coast is also known to be the site of frequent *Trichodesmium* blooms (Creagh, 1985). Far from these coastal areas, in the northwestern Pacific, eastern currents such as the Kuroshio Current or the equatorial counter current are sites of blooms in summer (Asaoka and Marumo, 1987). North of the Hawaiian Islands (at 28°N, 155°W in the North Pacific gyre) a *Trichodesmium* bloom was studied in detail by Mague *et al.*, (1974, 1977). In the southwestern Pacific, Baas-Becking (1951) reported observations of blooms around New Caledonia and the Fiji Islands and even at 25°S. A "gray tide" caused by *Trichodesmium* was reported by Bowman and Lancaster (1965) in the Tonga Islands, near 170° W, 10°S.

Visual observations of yellow waters by merchant ships' crews are available in the southwestern Pacific: between 1976 and 1982, the maximum occurrence of yellow stripes (supposedly formed by *Trichodesmium*) was between January and March, and northeast of New Caledonia. Dandonneau and Gohin (1984) suppose that the summer maxima of sea surface chlorophyll concentration at 165°E, 20°S are due to *Trichodesmium* blooms. Dessier (1988) explains the dominance of the Copepod *Macrosetella gracilis* (Harpacticoïda) around the New Caledonia-Vanuatu-Fiji archipelago by its well-documented association with *Trichodesmium*. Airborne observations in the oceanographic region around New Caledonia and Vanuatu confirm the fact that streaks forming "yellow tides" are typically found, between September and March, at convergence fronts in the northeastern region of New Caledonia (Petit and Hazane, 1982, Petit and Gohin, 1983).

Around the corallian Loyalty Islands, ten PREFIL (Production and Islands Effect) cruises were made between 1982 and 1984 (Le Borgne, 1986, map on Figure 1a). The positions of the fixed stations during the eight cruises (P3 to P10) are indicated. The presence of *Trichodesmium* was confirmed at all seasons, and all stations, between depths of 0 and 100 meters (Le Borgne, 1986). Figure 1b shows the visual observations of the occurrence of *Trichodesmium* puffs and tuffs forms, made from sieved water collected at various depths with a 30-liter Niskin bottle (Le Borgne, unpublished results; two abundance indexes are available). A vertical migration of *Trichodesmium* colonies is visible. Yellow streaks were indeed observed between Lifou and Ouvea islands, in May 1984, when *Trichodesmium* occurrence was maximum. Inside the lagoon, red tides are common, mainly on the northeastern coast, although there is no published report on this phenomenon.

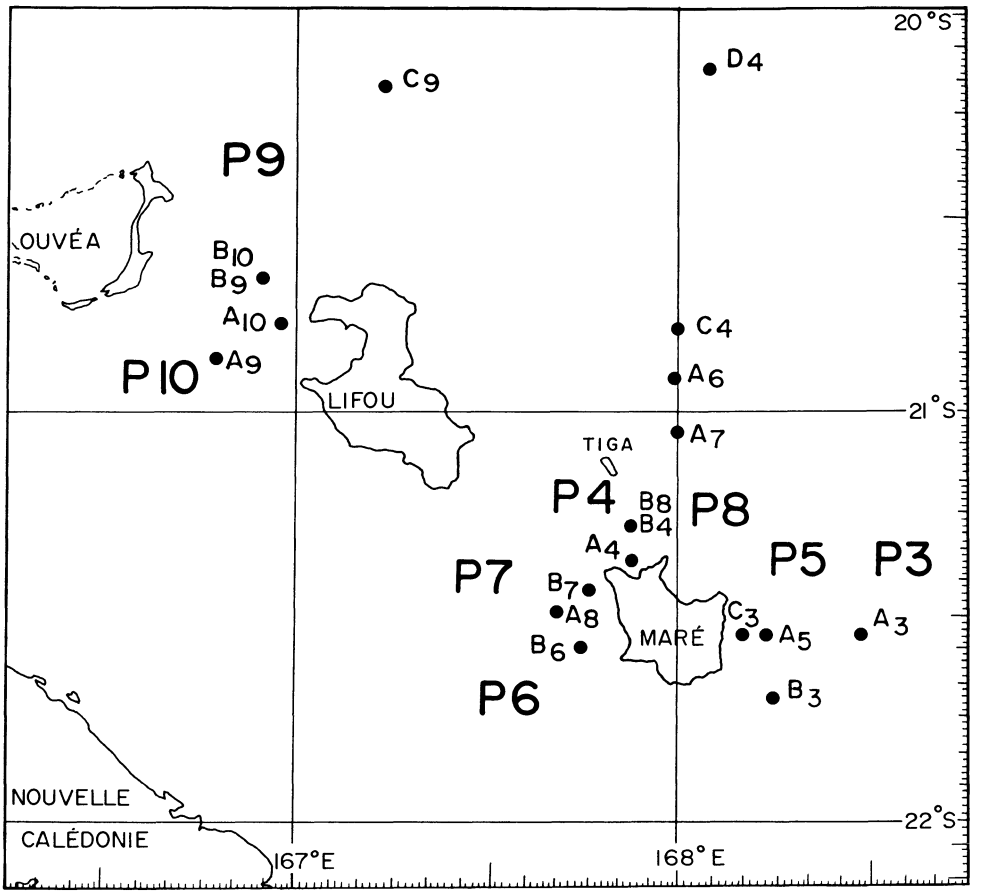


Figure 1a. Location of the fixed stations of the PREFIL cruises around the Loyalty Islands Maré, Lifou and Ouvea (Le Borgne, 1986). The cruises were undertaken in:
P3 : January-February 1982; **P4** : February 1983; **P5** : April 1983; **P6** : September 1983;
P7 : November 1983; **P8** : February 1984; **P9** : May 1984; **P10** : September 1984.

In April 1988 and November 1989, transects along 165°E confirmed that *Trichodesmium* was consistently found from 16°S to 8°S, on the entire water column and in surface waters where it caused a chlorophyll maximum (Blanchot *et al.*, 1988, Le Bouteiller *et al.*, 1991). *Trichodesmium thiebautii* was found at 15°S, and remained present for one week in November 1989 (Blanchot, personal communication). It is unknown within the equatorial region (Le Bouteiller, personal communication). For historical reasons, only a few references exist for the open waters of the southwestern Pacific. Nevertheless, it appears that in this area *Trichodesmium* blooms are a common phenomenon.

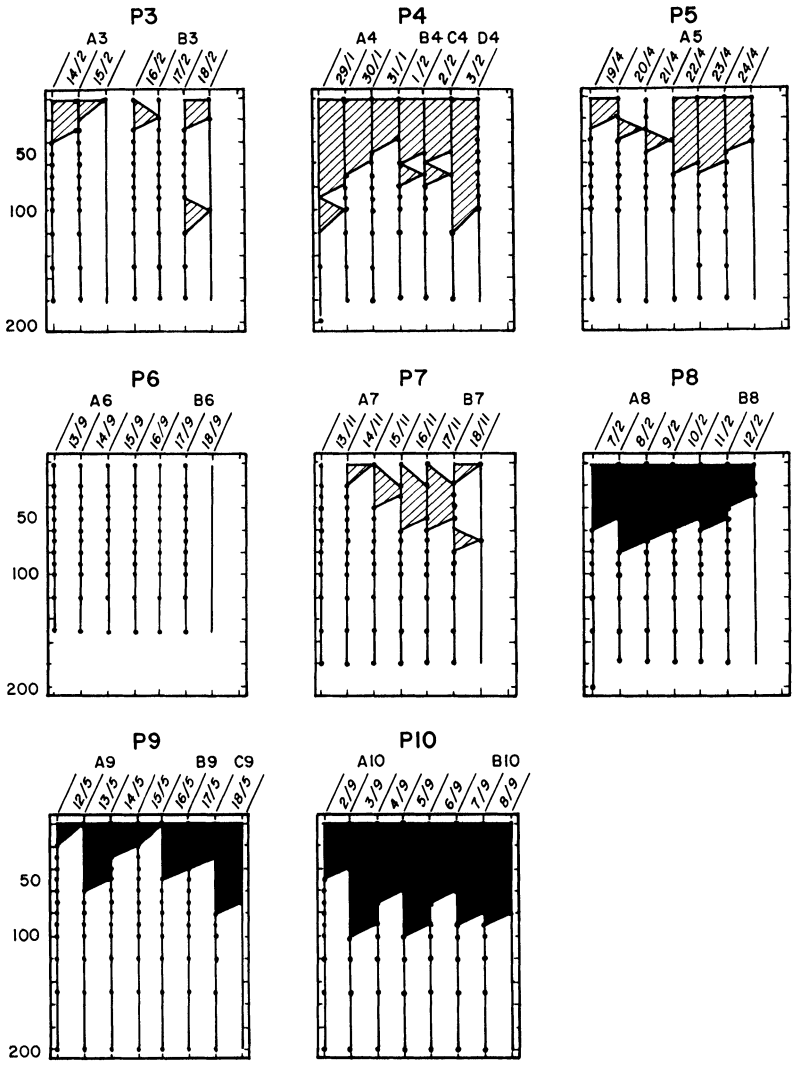


Figure 1b. Vertical distribution of the presence of *Trichodesmium* colonies observed at the fixed stations, as seen from samples collected with a 30-L Niskin bottle, and sieved on a 200-micron mesh. Two indexes of abundance are available. Vertical profiles are identified by date and by station number.

3. Bio-optical characteristics of oligotrophic and *Trichodesmium*-rich waters

A reflectance (R) model of marine waters has been developed by Morel (1980), which helps in computing for each wavelength the value of R as $0.33\ b_b/a$, where b_b and a are respectively the backscattering and absorption coefficients of the water body. The influence on *Rsat* of sediment and phytoplankton concentrations has also been modellised (André and Morel, 1991). In turbid waters, the sediment charge increases reflectance at all wavelengths due to an increase of backscattering (Viollier and Sturm, 1984). With very high concentrations of phytoplankton, green and yellow reflectances increase, blue strongly decreases due to chlorophyll absorption, and red slightly increases due to biological turbidity, as has been seen on the CZCS images of the Senegalese upwelling (Dupouy and Demarcq, 1987). According to Morel's model, when chl *a* varies from 0 to 0.25 $mg.m^{-3}$, green and yellow reflectance values are expected to vary from 0.9 to 1.5 %; blue reflectance between 3 and 1.5 %, and red reflectance from 0.06 to 0.17% (see Table 2 for reference values). According to this model, blue oligotrophic waters give almost no signal in the red.

Spectral reflectance measurements made over *Trichodesmium*-rich waters by Borstad *et al.* (1989) with concentrations varying between 100 and 1400 filaments.$liter^{-1}$ show striking features, mainly: a general increase of reflectance values at all wavelengths and four major dips in the spectra, corresponding to phycourobilin (495), phycoerythrin (625) and chlorophyll a (440 and 670) absorption peaks (Lewis *et al.*, 1988, Haxo *et al.*, 1987, Ohki *et al.*, 1986). High reflectance observed in the near infra-red (after 700 nm) is similar to the response of the terrestrial vegetation (Gausman, 1974). A high concentration of humic acids may follow *Trichodesmium* blooms (Jones *et al.*, 1986). Though these dissolved acids ("yellow substances") mainly absorb in the UV (Bricaud *et al.*, 1981), their absorbance may be large at 443 nm (Carder *et al.*, 1989).

The range of values for chlorophyll concentration around New Caledonia and the Loyalty Islands was observed to be 0.05 - 0.3 $mg.m^{-3}$ (Lemasson et Crémoux, 1985; Dandonneau and Gohin, 1984). Since the higher values were not common, those waters were assumed to be oligotrophic.

4. CZCS and SPOT observations

4.1. DATA

Observing a CZCS time series (60 images) taken from 1979 to 1984 has enabled us to describe the spatial and temporal distribution of discoloured waters. Dates and conditions of satellite acquisition are summarized in Table 1. CZCS images taken during summer (December to March) are often taken during sun glint conditions (despite the ability of the CZCS to avoid solar specular reflection by tilting). This may enhance the visible signal in a geometrically defined area and may alter the chlorophyll algorithm in that area only. Nevertheless, spectral signatures have been extracted in zones removed from these areas. SPOT images over the lagoon were also taken in sun glint conditions. In this case also, our observations were made outside these zones.

4.2. CZCS AND SPOT SENSOR PERFORMANCES

The CZCS sensor (Hovis *et al.*, 1980) has four bands in the visible domain: the blue (443 nm),

TABLE 1. Satellite observations in the 4 CZCS bands of NIMBUS-7 images of the Melanesian archipelago.

Months CZCS	Glitter	Channels 520,550	Channel 670	Channel 440	Chl *a* $mg.m^{-3}$
Nov 78*	-	+	+	+	0.11
Mar 79*	-	-	-	-	0.27
Apr 79*	-	-	-	-	0.12
Jun 79**	-	-	-	-	0.15
Jul 79**	-	-	-	-	0.15
Sep 79*	-	-	-	-	0.13
Nov 79*	+	-	-	-	0.19
Dec 79**	+	+	+	-	0.20
Mar 80**	-	-	-	-	0.15
Apr 80*	-	-	-	-	0.16
Jul 80*	-	-	-	-	-
Aug 80**	-	-	-	-	0.30
Sep 80*	+	-	-	-	0.18
Nov 80**	+	+	+	-	0.16
Dec 80*	-	+	+	+	0.19
Fev 81***	+	+	+	+	0.18
Mar 81**	-	+	+	-	-
Apr 81***	-	-	-	-	0.18
May 81*	-	-	-	-	0.16
Jun 81*	-	-	-	-	0.19
Jul 81**	-	-	-	-	0.19
Aug 81*	-	-	-	-	0.22
Nov 81*	-	-	-	-	-
Jan 82**	+	+	+	+	0.14
Mar 82**	-	+	-	-	0.19
Apr 82**	-	+	-	-	-
May 82*	-	-	-	-	-
Jun 82*	-	-	-	-	-
Jul 82*	-	-	-	-	-
Oct 82*	-	-	-	-	0.19
Apr 83*	-	-	-	-	0.14
Sep 83*	-	-	-	-	0.14
Oct 83*	-	-	-	-	0.14
Mar 84*	-	+	+	+	0.14
Apr 84*	-	-	-	-	-
Aug 84*	-	-	-	-	0.14
Sep 84**	-	-	-	-	0.10
Oct 84*	-	-	-	-	0.10
Dec 84**	-	-	-	-	0.10
Month SPOT					
Dec 86 +	+	+			

green and yellow (520 and 550 nm) and red (670 nm). It also has a near infra-red channel at 750 nm used to discriminate land areas. The "penetration depth" corresponding to 90% of the satellite signal is maximum in the blue oligotrophic waters of the southwestern Pacific. Computed as the reverse of the attenuation coefficient from the PREFIL data around the Loyalty Islands and PROPPAC data (Le Bouteiller, personal communication), it varies from 16 to 25 meters (depending on the wavelength). Conversely, the red wavelength is totally absorbed by water and the signal then corresponds to depths of only a few centimeters. As a result, this channel is used to extract the atmospheric contribution from the total signal (Viollier *et al.*, 1980, Deschamps and Viollier, 1987). When an accumulation of algae occurs near the sea surface, the near infra-red signal may be high. It has been widely used to detect cyanobacterial blooms with high biomass levels or intense surface scums (Horstmann *et al.*, 1978, Galat and Verdin, 1989). The pixel size is 825 m x 825 m and the swath width is 1600 km.

The SPOT sensor has three wide bands centered at 550, 650 and 850 nm. The penetration depth is about 15 m for band 1, a few centimeters for band 2. The last channel is sensitive to the first few centimeters and mainly to the near infra-red reflectance of terrestrial vegetation ("red edge"). Pixel size is 20 m x 20 m, and swath width is 60 km.

4.3. CZCS AND SPOT SENSOR RESPONSES

Around New Caledonia and Vanuatu, except for lagoon waters and turbid river runoffs, there should be no signal in the 670 nm band. Following chlorophyll concentrations found by the CZCS (Table 1), the four-band spectra should be those of oligotrophic waters. This is mostly the case in the CZCS series, except in summer, where many images show well-organized bright linear patterns with relatively high reflectance in the red and green-yellow channels. On Table 1, signs (+) and (-) indicate the presence on the individual channel of reflectant waters. Chl *a* is the mean chlorophyll concentration of the CZCS image (1600 km x 900 km) with an accuracy of 30% (Hovis *et al.*, 1978). All images were atmospherically corrected using the concept of the "clear water" pixels (Gordon *et al.*, 1983) and only 2 Angström exponents (0.8 and 1). (*) represents the number of images available for the month. In the "true colour mode" (Viollier and Belbeoch, 1984), these bright waters appear red, gold-yellow, or even white on the CZCS images, when their blue reflectance is higher than for blue oligotrophic waters.

In Table 2, CZCS reflectance values in the four bands are compared for pixels chosen in different parts of the image: in the most reflectant rows, in blue clear waters, in chlorophyll-rich surrounding waters. In every case, the bright water reflectances differ from the model, for all bands. For January 82 and February 1981, all reflectance values are particularly high from the blue to the red bands. For December 1980, only green and yellow reflectance values are high. This variability in the blue reflectance shows that "red tides" can have a variable CZCS signature in the Melanesian archipelago. They never reach the high CZCS reflectance values of coccolithophorid-rich waters where calcareous plates increase the backscattering of light (Viollier and Sturm, 1984, Viollier *et al.*, 1988). The effect of the phycoerythrin pigment is slightly detectable as the reflectance is generally lower at 550 nm than at 520 nm.

SPOT numerical counts of pixels in the streaks show that reflectances are lower than those of the coral barrier reef, but are much higher than those of the blue surrounding waters. The signal is higher in the first two bands, but comprehensive atmospheric corrections will be necessary before this can be confirmed. The SPOT image was taken in December 1986, just after the tropical storm "Patsy" (Territorial Meteorological Service, Noumea).

TABLE 2. Reflectances in % in the four CZCS bands for different water types selected on the image: blue water, rich water ($Csat = 0.3$ mg m^{-3}), bright water (detected white or yellow on the true colour mode, see text).

Water type	Band 1	Band 2	Band 3	Band 4
Reference: André and Morel, (1991)				
Blue water	3	0.9	0.9	0.07
Rich water	1.5	1.5	1.5	0.17
Coccolithophorids: Viollier and Sturm, (1984)				
Bright water	9-5	7-5	6-4	1.5-1
4 January 82				
Bright water	5-3.5	3.8-2.9	3.8-2.7	1-0.4
Blue water	3.2	1.2	1	0.2
Rich water	1.1	0.8	0.6	0.2
4 February 1981				
Bright water	5-4	5-3	4-2.1	1
Blue water	3-2	1.5	1.5	0.3
Rich water	2	1.5	1.5	0.3
27 December 1980				
Bright water	3-2.2	2.1-1.8	2-1.6	0.4
Blue water	3	1.2	1	0.2
Rich water	1.2	0.8	0.8	0.2

4.4. SPATIAL DISTRIBUTION

In almost all summer images, bright elongated streaks and meandering rows, a few kilometers large, and hundreds of kilometers long (Plate I) are detected. These patterns are similar in shape to those detected by the Space Shuttle and reported by Kuchler and Jupp, (1988) on the Great Barrier Reef, where surface streaks of a "red tide" presumably formed by *Trichodesmium* were trapped.

On January 4, 1982, the bright linear structure could be followed, along 400 km, in the axis of the chlorophyll-rich waters, from New Caledonia (165°E) to Vanuatu (170°E). Two days earlier, similar bright meanders could be followed from Vanuatu to Fidgi (180°E) and even to Tonga (175°W). This indicates that blooms can be followed along 10 degrees of longitude. In March 1984 as well as in February 1981, meandering rows covered the chlorophyll-rich image, between 170°E and 175°E. In December 1980, the "red tide" was found on the exact border of the green waters. In the other images, well-organized gyres were restricted to smaller areas, as in November 1978, November 1980, December 1979 and March 1982. The advantage of the CZCS is that it gives two items of information simultaneously: one integrated over 20 meters in the blue band, and one for the surface in the red band. The chlorophyll-rich waters can reach 90 000 km^2, while bright linear patterns represent only 10 per cent of this surface. Comparison of the two bands for the same image gives a complete set of useful information on these discoloured waters. This is well shown on Figure 2.

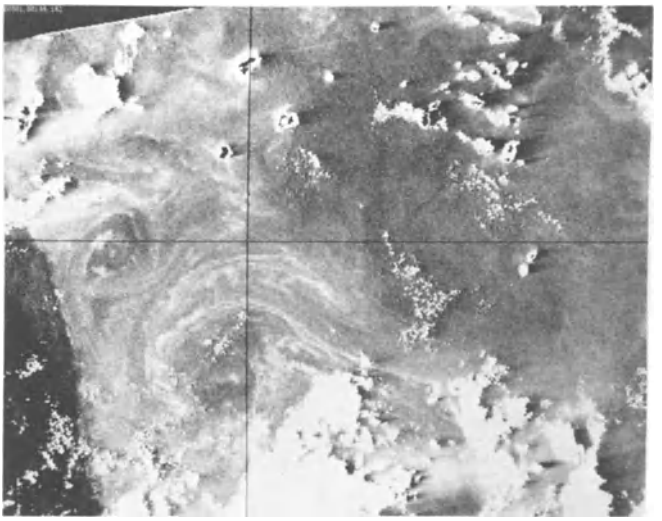


Plate I. CZCS image of a bright meandering pattern off the southern Fidgi Islands at 20°S, 180°E. Enhanced channel 3 (550 nm). Meanders are a few pixels wide. 2 January 1982.

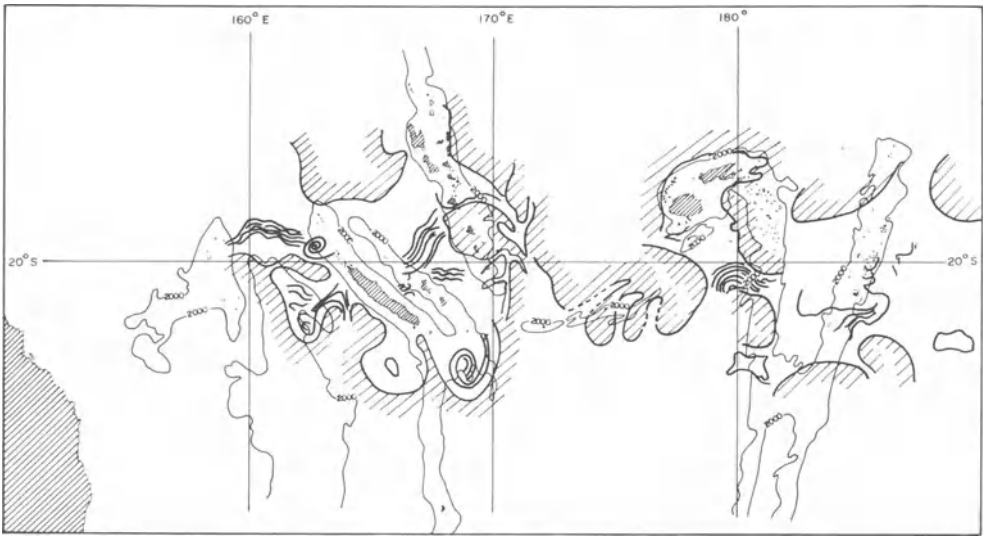
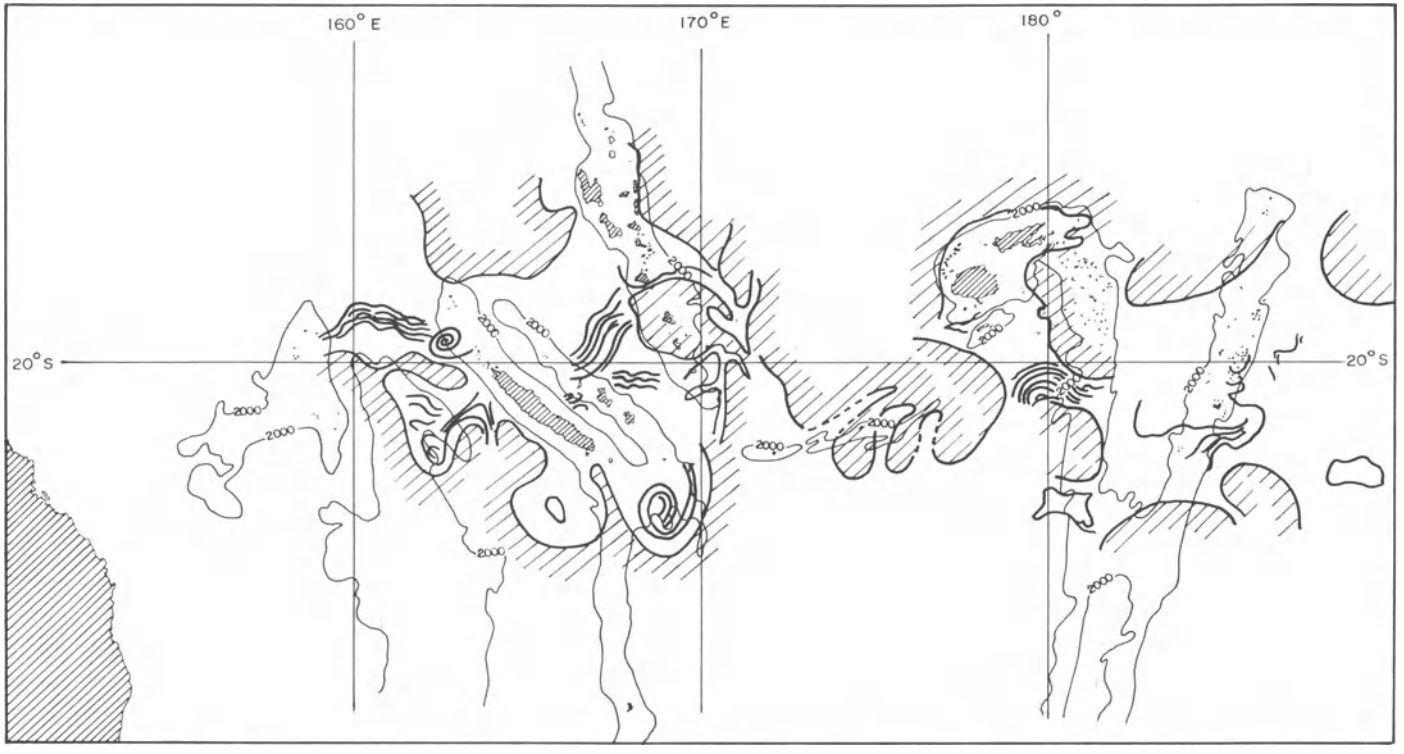


Figure 2. Area of discoloured waters observed by NIMBUS 7 Coastal Zone Colour Scanner around New Caledonia, Vanuatu and Tonga. Schematic mapping of chlorophyll-rich waters (inside of the hatched areas), with an outline of distinguishable bright linear patterns. West of 180°E, mosaic of images taken on several dates (November-December 1979, December 1980, February 1981, 2-4 January 1982, March 1982, March 1984). East of 180°E, one image taken on 2 January 1982.



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The SPOT images taken in December 1986 in the lagoon waters off St. Vincent Bay (Plate II, west coast) and off Canala (east coast) show white lines and eddies. They are one or two pixels wide and a few kilometers long. These patterns are found parallel to the coast, but perpendicular to the wave direction, and resemble cyanobacterial streaks observed by previous authors.

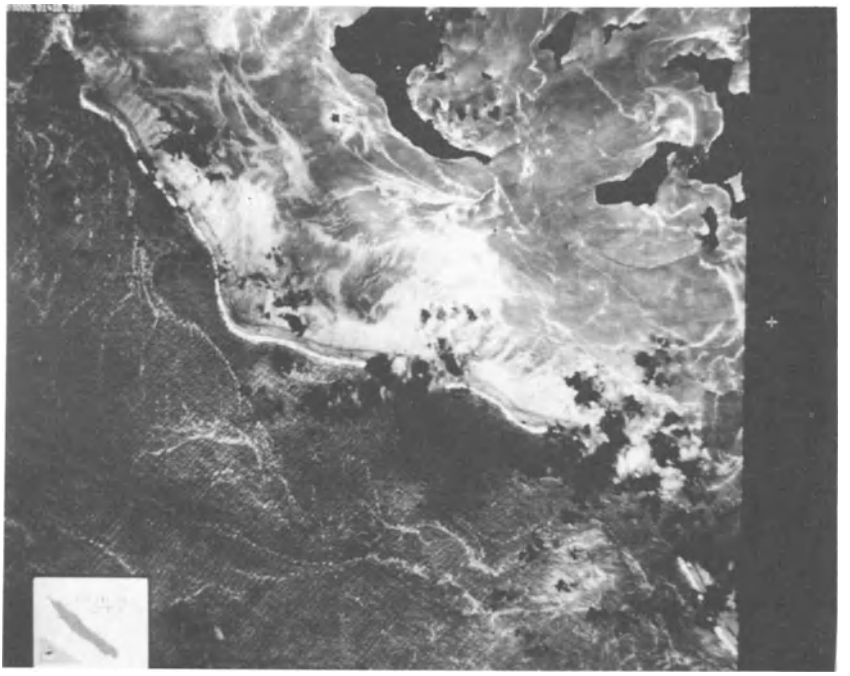

Plate II. C Copyright SPOT 1986 under license SPOT Image. Image of St. Vincent's Bay (west coast of New Caledonia). Enhanced XS1 channel. The figure represents a surface of 20 km x 20 km. The area of the pixel is 20 m x 20 m. Characteristics of the image: orientation: 8°6, incidence: 13.8°, solar azimuthal and zenithal angles: 98.2° and 68°3. Clouds and land are masked in black. Bright streaks are 20 meters wide. They appear around the small islands inside the coral barrier (white), as well as outside it, where they appear to be disrupted by waves.

5. Discussion and conclusion

CZCS and SPOT spectra show high reflectances over the entire spectrum. These high reflectance values have been discussed by Dupouy *et al.* (1988). They are explained by a highly diffusive biological phenomenon due to mass accumulation of algae. *Trichodesmium* colonies are known to form dense windrows of white to brown coloration at the sea surface. The CZCS spectra measured *in situ* by Borstad *et al.* (1989) on concentrated colonies corroborate this assumption. Other cyanobacteria are known to be part of the phytoplankton of archipelago waters. Picoplanktonic cyanobacteria dominate the phytoplankton in the southwestern Pacific (Blanchot *et al.*, in press), but do not form diffusive streaks. Coccolithophorids are a major group in the Caledonian lagoon (Desrosières 1969, 1975). They are known to form very intense white patches of highly diffusing coccolith plates (Le Fèvre *et al.*, 1983; Holligan *et al.*, 1983; Viollier and Sturm, 1984; Viollier *et al.*, 1988). CZCS-derived spectra over bright Pacific waters never reach such values. The effect of phycoerythrin is not as clear as expected on these CZCS examples and requires further study (Subramanian, unpublished results).

The examination of the 60 images of the series confirms the conclusion of Dupouy, (1990a,b). The majority of the summer images show high chlorophyll concentration zones and distinguishable patterns. These patterns corresponding to "red tides" are not systematically bright as for February 1981, January 1982 or March 1984, despite the probable presence of *Trichodesmium*. This variability of CZCS signatures is plausible, as the state of *Trichodesmium* blooms can vary with time and oceanographic conditions. The comparison of the blue and red bands of the CZCS is meaningful in this sense. The percentage of floating and buoyant colonies is given by the red band image, while the total bloom surface is given by the blue band. Present SPOT or NOAA satellites lack the blue band and therefore can describe only the surface of the bloom. The SPOT images of the Caledonian lagoon were taken after a tropical storm. Heavy rains may have caused extensive land water runoffs. On the other hand, *Trichodesmium* blooms are known to occur after tropical storms (Petit, personal communication). Patterns are likely those of *Trichodesmium*. It must still be confirmed that *Trichodesmium* is responsible for the extensive chlorophyll increases as well as for the "bright red tides", and that no other phytoplanctonic species can form the same patterns.

The circumstantial evidence in favour of *Trichodesmium* blooms is high. The discoloured waters with red tide signatures are commonly found during the austral summer (December to March), or after calm spells. The oceanographic context is favourable: off eastern New Caledonia, a year-round convergence zone is known to occur between the Trade Drift zone and the west-flowing southern equatorial current (Rotschi and Lemasson, 1967). High sea temperatures from January to March are favourable to *Trichodesmium* blooms.

Western Pacific discoloured waters are well documented by the CZCS. Their origin must still be confirmed by *in situ* observations extended to the whole Melanesian archipelago. The diazotrophic *Trichodesmium* is the most probable cause of such blooms, according to Loyalty Islands field measurements. If so, they constitute a major event for nitrogen fixation (Capone and Carpenter, 1983), and organic matter input in the oligotrophic waters. Because of the permanent stratification of these waters, nutrient inputs are reduced; the ecological impact of such nitrogen-fixing blooms may therefore be large.

Because of better repetitivity and oceanic global coverage, additional channels (mainly at 495 nm for phycourobilin pigment), and the probability of improved atmospheric corrections and radiometric resolution, future ocean color sensors will help in more confidently detecting *Trichodesmium* blooms. Airborne and *in situ* radiometric measurements will still be fully necessary in order to determine the evolution of the optical properties of blooms, which are undoubtedly related to concentration, age and the state of *Trichodesmium* colonies. Even before bio-optical studies are undertaken, better documentation on bloom occurrences of *Trichodesmium* in the region and over the global ocean will be essential.

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DEVELOPMENT OF ALGORITHMS FOR REMOTE SENSING OF *TRICHODESMIUM* BLOOMS

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ABSTRACT. *Trichodesmium* is a major component of the global carbon cycle, but because of its sporadic occurrence it is extremely difficult to study by conventional shipboard methods. Information on the variability and spatial extent of this cyanobacterium is essential for calculation of its contribution to carbon and nitrogen fluxes. Intense surface blooms of *Trichodesmium* have been observed in satellite imagery from the Coastal Zone Color Scanner and in color photography from the space shuttle, but such reports are rare. To date it is difficult to differentiate *Trichodesmium* from other species by remote sensing measurements alone.

A consideration of the spectral reflectance and absorption measurements on natural and concentrated populations of *Trichodesmium* shows that at moderate concentrations, *Trichodesmium* and other cyanobacteria should be distinguishable from diatoms and dinoflagellates where high spectral resolution data are available. This paper discusses optical data collected from freshly collected *Trichodesmium*, focussing on narrow spectral absorption features resulting from the nitrogen containing pigments at 495 and 545 nm by phycoerythrin, and at 625 nm by phycocyanin. The specific absorption spectra are used in an optical model to generate reflectance spectra corresponding to different concentrations of *Trichodesmium*. The detection limits of algorithms based on these features are assessed. The model spectra are also compared to actual reflectance data from a series-dilution experiment. This treatment illustrates the potential to use existing and planned airborne and space craft water color sensors to map *Trichodesmium* and other cyanobacterial blooms.

1. Introduction

The cyanobacterium *Trichodesmium* spp (= *Oscillatoria*) is the most abundant and active nitrogen fixing species in the plankton of tropical and sub-tropical seas (Carpenter, 1983). The organism plays an important role in the global flux of nitrogen and carbon. However, its appearance in infrequently travelled tropical seas far from major research laboratories, combined with the difficulty of keeping it alive in culture, has made it difficult to study.

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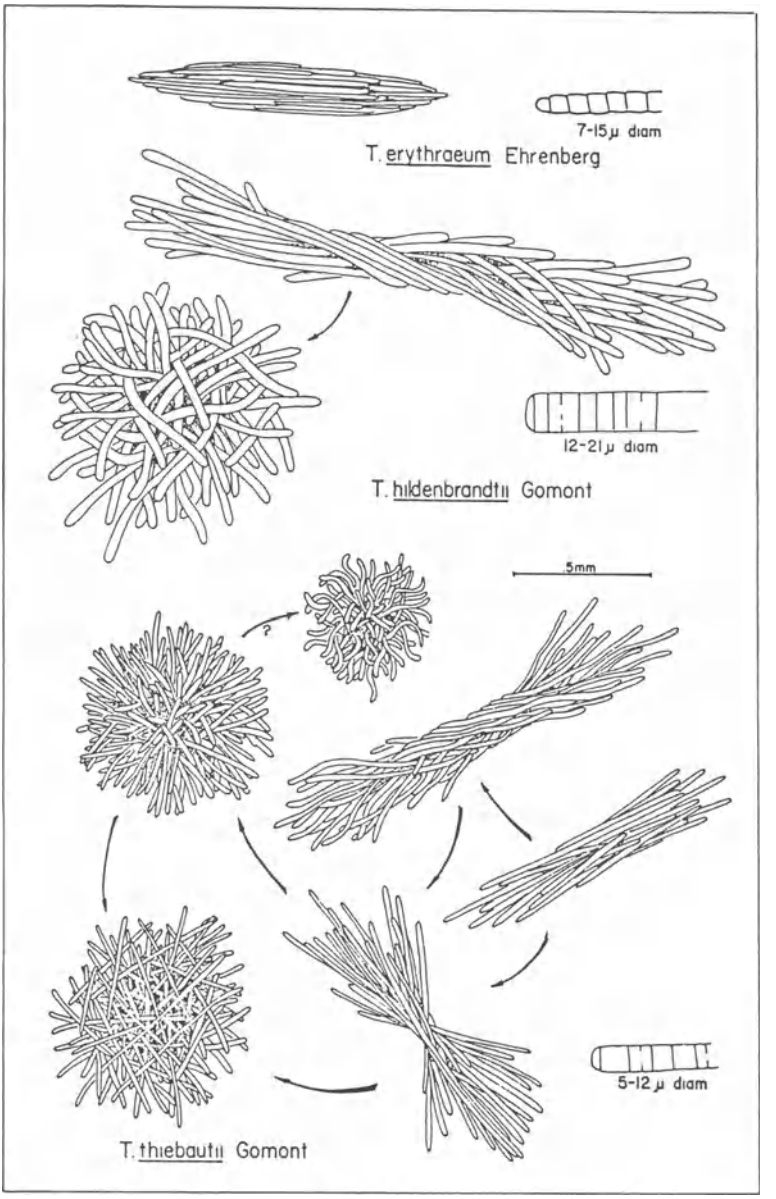

Figure 1. The colonial forms and relative sizes of Trichodesmium spp., (with speculations concerning relationships between forms; from Borstad, 1978).

Trichodesmium is non-uniformly distributed on all space scales. At the largest scales, the organism can form massive concentrated blooms stretching over hundreds of kilometers (Devassy et al., 1978). Gas vacuoles cause it to float on or near the surface, and convergence in the surface layers of the ocean can concentrate blooms into linear surface slicks. At the smallest scales, some variable and unknown fraction of the population of individual filaments (trichomes) of 100 or so cells (of 5 to 10 μm in length) are concentrated into colonies of tens to thousands of trichomes (figure 1). This colony-forming characteristic will also cause its concentration to be underestimated in remote sensing measurements.

The global distribution of the species, frequency of blooms and estimates of rate of N_2 fixation and primary production could be assessed more accurately by the use of remote sensing, especially if quantitative methods can be developed which differentiate *Trichodesmium* from other phytoplankton. Similarly, differentiation of cyanobacteria from other bloom-forming species in fresh water bodies would also be a significant improvement to present capabilities. In this chapter we discuss preliminary measurements of the organism's *in vivo* optical properties, made on artificially concentrated samples. The chlorophyll specific absorption spectra are then used to model reflectance spectra of *Trichodesmium* and a reference diatom species. Reflectance spectra are also compared to measurements made on serial dilutions of artificially concentrated *Trichodesmium*.

Trichodesmium contains both chlorophyll a and the bilin pigments phycoerythrin and phycocyanin which have characteristic absorption spectra. These features should become visible in remotely observed spectra at some cell concentration. Our studies were undertaken with a view to determining the detection limits for these features and, in general, to prepare for the higher spectral resolution remote sensing instruments now becoming available. The present family of space-borne water-color sensors is not optimal for this purpose, but as will be discussed below, improved sensors are being launched in the next decade.

2. Observations of *Trichodesmium* from Space

The Coastal Zone Color Scanner (Hovis et al., 1980) collected global data on near-surface phytoplankton biomass during its lifetime from 1978 to 1986. The CZCS was a mechanical scanner with a spatial resolution of about 1 km that made high sensitivity measurements of water-leaving radiance in four bands, each 20 nm wide, centred at 443, 520, 550 and 670 nm. Algorithms for deducing chlorophyll concentrations from ratios of blue and green radiance values have been developed and tested (Gordon et al 1983). With correction for atmospheric effects calculated from the longer wave bands and under so-called "Case 1" conditions in which the optical properties of the water are dominated by the presence of phytoplankton (Morel and Prieur, 1977), the algorithms have been shown capable of accuracy to better than a factor 2.

However, the algorithms were calibrated using more accessible diatom and dinoflagellate species. There have been few reported cases in which *Trichodesmium* was the dominant species in the water, and where ship measurements were made of its concentration at the time of a CZCS overpass. Moreover, the colonial behaviour of *Trichodesmium* suggests that self-shading may reduce the effect of pigments present on the optical properties of the water. Exact calculation of this effect requires knowledge of the effective optical depth of the pigment in a single cell (including increase of the path length in the cell by scattering), and a good statistical description of the clustering properties of colonies. At low cell numbers however, water color is dominated by absorption by water itself and by chlorophyll a. In spite of gas vesicles and auxiliary pigments, single cells of *Trichodesmium* are probably sufficiently similar to diatoms and

dinoflagellates that the CZCS algorithms could be expected to apply if the individual *Trichodesmium* cells were dispersed.

However, pigment in colonies may be seriously underestimated. If it is assumed that the optical depth of the pigment in a cell is significant, i.e of the order of unity or greater, then clustering of cells results in some being shadowed by others and an overall reduction of light absorption for a given pigment concentration. The gathering of about 100 *Trichodesmium* cells roughly 10 μm across into linear chains, trichomes about 1000 μm long, should not result in significant shadowing, since the cell axis will on average make an angle of 60 degrees to the direction of incoming and outgoing photons. The 100 cells would still have roughly their entire area 100 x 10 x 10, or 10^4 μm^2 , available for intercepting light.

Clustering of the trichomes into colonies can result in a more significant shadowing. If 10,000 trichomes were stacked evenly to form a cube 1,000 μm on a side, then shadowing would be by a factor of 100. That is, the area available to intercept light would be 10^6 μm^2, equivalent to that of 100 trichomes, instead of to the 10,000 trichomes actually present.

The actual clustering of trichomes is much more random and variable, roughly falling into two configurations: "radial, or puff" and "parallel, fusiform or tuft" forms (figure 1). The colony nomenclature differs between authors. A factor of 3-10 for the effect of shadowing appears a reasonable assumption. This effect will seriously reduce visibility of moderate concentrations of *Trichodesmium* in CZCS surveys.

However, the gas vesicles in *Trichodesmium* tend to increase its visibility, since they can bring the alga to the surface where blooms may accumulate under appropriate conditions. Marine and fresh-water cyanobacterial blooms have been well studied, but have not been the subject of much remote sensing effort to date. There are only three papers describing detection of blooms of fresh water cyanobacteria with the multi-spectral scanner on the LANDSAT series satellites. Horne and Wrigley (1975) reported early studies of *Anabaena* and *Microcystis* blooms using the near infra-red bands. Horstmann et al. (1978) and Ulbricht (1983a, b) have detected *Amphanizomenon* and *Nodularia* blooms in the Baltic, also using LANDSAT MSS band 6. In the late 1980's, there have also been two papers discussing remote sensing of *Trichodesmium* blooms from space. Kuchler and Jupp (1988) presented a natural color photograph taken from the US Space Shuttle using a hand-held camera, showing a massive *Trichodesmium* bloom off the coast of Australia. DuPouy et al, (1988; and this volume) have shown an example of a bloom imaged by the Coastal Zone Color Scanner at a time when high concentrations of *Trichodesmium* are common in surface waters near New Caledonia. In their example of a surface scum, the CZCS shows an increase in radiance in all bands, but with a smaller increase in the blue band at 440 nm.

We have studied a series of CZCS images collected in the spring of 1979 covering the west coast of India during March, during the time period (February to May) when Devassy et al (1978) report regular occurrence of *Trichodesmium* blooms along a coastal strip normally between the 5 m and the 25 m isobaths, with more detailed observations in 1977 and 1978. Devassy notes that this pre-monsoon season is characterized by clear skies, low winds and little coastal runoff. The CZCS images cover large cloud-free areas stretching 1500 km offshore and about 800 km along the coast centred on the region from Bombay to Goa. The images show a persistent pattern which is well illustrated by figure 2. Over most of the area the radiance back-scattered to the satellite at 520 and 550 nm is low. This is the type of water referred to as Case 1 by Morel and Gordon (1983). Radiance variations at 443 nm show the effects of absorption by varying amounts of chlorophyll pigment, and the chlorophyll image in figure 2d shows the quantitative pigment concentrations which should be accurate to within a factor 2 for common phytoplankton species.

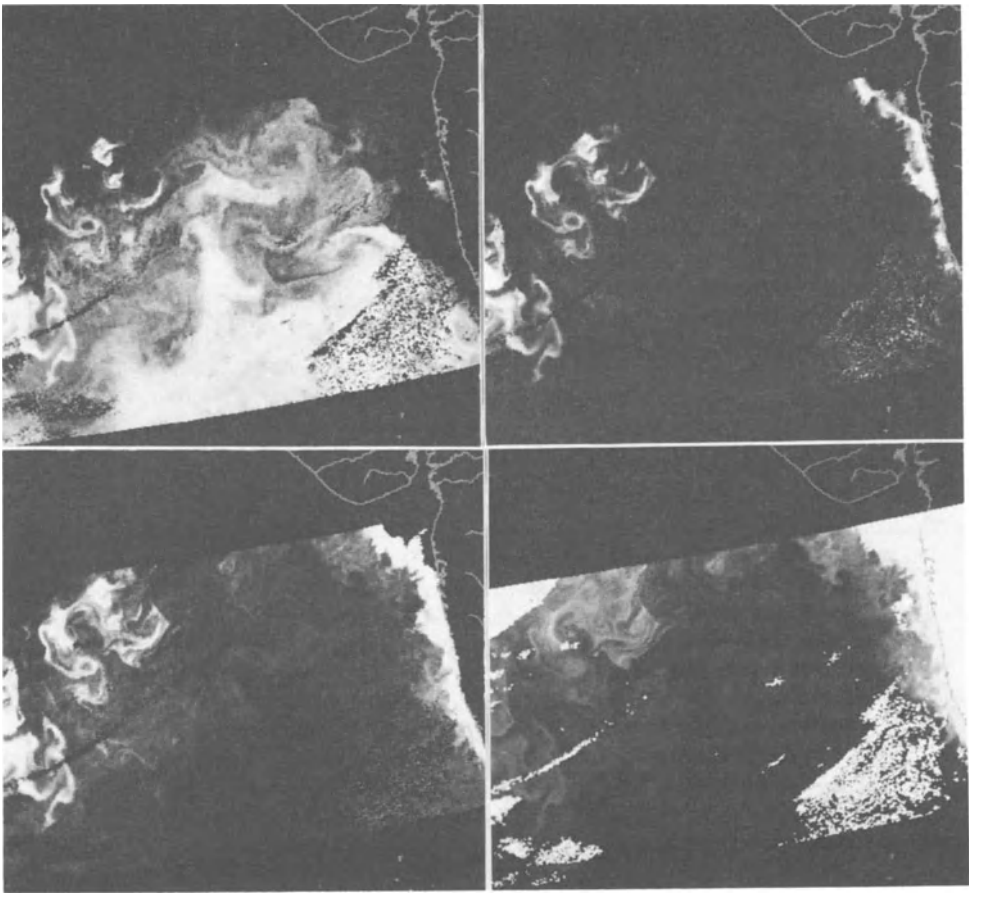

Figure 2 (a to d). CZCS images of water-leaving radiance at 443 nm (upper left), 520 nm (upper right) and 550 nm (lower left) and chlorophyll pigment distribution (lower right) in the Arabian Sea for March 29 1979.

Close to the coast of India, higher radiances are observed in the 520 and 550 nm bands, while in the 443 nm band, reduced radiances are observed, implying high pigment concentrations with high back-scatter. This is the type of signature that should be expected for a *Trichodesmium* bloom. In such "Case 2" waters the chlorophyll pigment concentrations may not be accurate, and pigment concentrations due to *Trichodesmium* will tend to be underestimated as noted above. The region of high radiance in figure 2 covers an area of about 20,000 km^2 over the continental shelf of western India. These case 2 waters extend into deeper water than reported by Devassy for the occurrence of *Trichodesmium*. However, the accuracy and coverage of Devassy's surveys are not clear. Without further in-situ measurements it is not possible to definitely identify the source of the scattering, but the coincidence is suggestive.

Reduced radiances at 433 nm are observed in Figure 2a to distances farther from the coast of India than the region of high backscatter at 520 and 550 nm. The additional area of elevated pigment values (compared to farther offshore) is about 7,000 km^2, and may be related to fertilization via nitrogen fixation by the *Trichodesmium* closer to shore.

On the left side of Figure 2 are distinct regions, covering a total of about 10,000 km^2, showing high radiance in all 3 CZCS bands at 433, 520 and 550 nm. This is the signature expected from a strongly-scattering bloom such would be caused by coccolithophores, which are known to be common in this area.

From this example it can be seen that CZCS images can be useful in determining the area of intense blooms. Alternatively, improved airborne senors are available now. While satellite sensors have relied on broad spectral bands to image the scattering or chlorophyll absorption, new technology is making available sensors with higher spectral resolution (Borstad et al., 1985; Vane, 1987), which in combination with studies of the pigment specific absorption properties of different classes of alga promises to significantly advance remote sensing of phytoplankton through increased pigment differentiation, pigment ratios and chlorophyll fluorescence.

3. Optical Measurements

While there are a few relative *in vivo* absorption spectra for *Trichodesmium* in the literature (Shimura et al., (1975), McCarthy and Carpenter, (1979), and Ohki et al., (1986), there is only one report of chlorophyll specific absorption coefficients (Lewis et al., 1988) which are required for optical modelling. No information has been available concerning the reflectance spectrum of the cyanobacterium and how this changes with changing pigment concentration.

We made optical measurements of above-water reflectance, in water vertical profiles of downwelling radiance and of absorption by isolated and concentrated *Trichodesmium* colonies on cruise number 18 of the University of Miami vessel RV Columbus Iselin, between November 4 and November 24, 1988. The cruise track, from Miami through the Bahamas to the Honduran Island of Roatan, and on to Limon, Costa Rica crossed a wide area of the Caribbean where *Trichodesmium* occurs at relatively low concentrations and where blooms have been observed. Several interrelated experimental programs were also conducted, providing supporting measurements of carbon and nitrogen fixation, nutrient uptake and cell counts.

3.1. SPECTRAL ABSORPTION COEFFICIENT

Individual *Trichodesmium* colonies were picked out of the contents of a surface plankton tow using pipettes, washed through filtered sea water, and dispersed in small volumes of filtered water by mechanical stirring. The suspension of trichomes was then collected on moist GF/F filters papers. Absorption was measured according to the method of Mitchell and Kiefer (1988), using the Institute of Ocean Sciences spectrometer (Walker et al 1975) looking through the dispersed colonies on GF/F filter paper at an Osram halogen bulb. Experiments on serial dilutions of the same sample showed that Mitchell and Kiefer's Beta factor could be applied to our samples, since all serial dilutions gave within 5% absolute absorption when normalized by the ratio (sample volume/filter area of the filter) and Beta. Beta is the so-called 'path length amplification factor', which is defined as the ratio of absorption of cells on a filter paper, which itself is highly scattering, to the same number of cells in a minimally scattering suspension. Bricaud and Stramski (1990) have also confirmed the applicability of Mitchell and Kiefer's (1988) Beta factor where optical densities of the sample are greater than about 0.20. Figure 3 shows typical chlorophyll specific spectral absorption coefficients (a_{ph}) for dispersed trichomes

of *Trichodesmium* (probably *T. theibautii* and *T. hildenbrandtii*), in this case for station 14 in the Eastern Caribbean at approximately 17° 01.8' N 82° 08.2' W.

Our spectrum shows a maximum absorption peak at 435 nm and a second peak at 675 nm, due to chlorophyll a. Peaks at 494 nm and 541 nm are ascribed to phycoerythrin and that between 619 and 624 nm to phycocyanin. Our spectra are of similar shape to the relative absorption spectra for *Trichodesmium* reported by Shimura et al., (1975), McCarthy and Carpenter, (1979), and Ohki et al., (1986), but we did not observe the peak at 565 nm (phycoerythrobilin chromophore) as reported by Shimura et al., 1975 or Ohki et al (1986) for *T. erythraeum*. Our spectra are very different both in shape and absolute value from those reported by Lewis et al., (1988) from the western Sargasso at 35° 35'N 65° 45'W. Lewis' spectra show very low absorption across the 500 to 600 nm region (perhaps related to a phycoerythrin deficit relative to chlorophyll), and have not been normalized to zero at 750 nm. His samples were collected by reverse filtration from a bucket from a concentrated bloom while ours were picked individually out of the contents of a plankton tow.

Variations in chlorophyll specific absorption in other types of phytoplankton have been found to be related to senescence (Kiefer et al., 1979); growth irradiance (Dubinsky et al., 1984) as well as cell size, intercellular pigment concentration and presence of auxiliary pigments (Sathyendrenath et al., 1987). How much of the difference between our measurements and those of others is due to pigmentation and physiological differences and how much is due to variation in technique is unclear. More detailed investigation of these differences is needed.

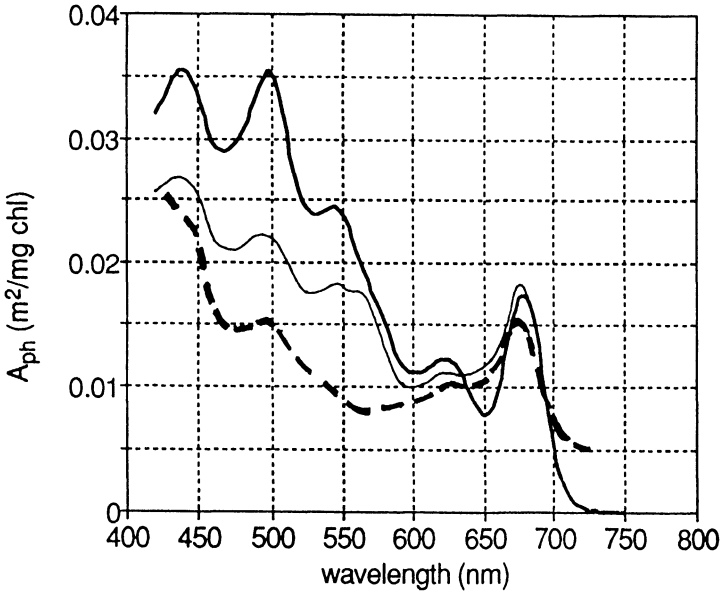

Figure 3. Chlorophyll specific spectral absorption coefficients (a_{ph}) for dispersed trichomes of *Trichodesmium* from the Caribbean (heavy line), compared to a_{ph} reported by Lewis et al (1988) for *Trichodesmium* from the Sargasso (dashed line) and a relative absorption of *Trichodesmium* from the Kuroshio, normalized to the 670 nm peak (Ohki et al., 1986).

Our absolute value at the 440 nm absorption peak (.035 m^2mg^{-1} chlorophyll m^{-3}) falls within the range reported by Mitchell and Kiefer (1988) for various cultures, especially those grown at high light intensities, and within the upper part of the range shown by Sathyendranath et al. (1989) for cultures of diatoms and dinoflagellates. It is lower than reported by Bricaud et al., 1983, and by Morel and Bricaud (1981) for *Coccolithus huxleyi*. Our values do not agree with the absolute absorption coefficients published by Lewis et al. (1988), especially below 600 nm. Lewis used a constant value of 2.45 for Kiefer's Beta term which describes the optical path length amplification caused by scattering (Kiefer and Soohoo, 1982; Mitchell and Kiefer, 1988) and does not seem to have normalized absorption to zero at 750 nm.

Lewis et al. measured a decrease of photosythesis by about a factor 3 at high light in *Trichodesmium* samples which were mechanically disrupted, compared to samples with intact colonies. They concluded that this was due to an increase in the diffuse attenuation coefficient of the algae *in situ* because of the self shading which would normally be present due to colony formation. We noted above that self shading of this order of magnitude might be expected. In one case we measured a 2 to 4 times increase in attenuation when intact colonies suspended in seawater in an Utermöhl cell were dispersed. However, this was for a very high concentration (7785 trichomes ml^{-1}) so that Beers law does not apply (Sathyendrenath et al., 1987).

3.2. MODELLED REFLECTANCE SPECTRA

3.2.1 *The model.* A simple model of the spectral irradiance reflectance was proposed by Morel and Prieur (1977), and has since been developed by Prieur and Sathyendranath (1981), (Morel 1980 and 1988), Gordon and Morel (1983) and Sathyendranath et al. (1989). The basic model is:

$$R_i = 0.33\, B_t/A_t \qquad (1)$$

where B_t is the total backscattering from the water and A_t is the total absorption in the water. Both B and A are due to water itself, to suspended material and to dissolved organics (known as Gelbstoff). All constituents make independent, additive contributions. When modified for the reflectance factor observed in remote sensing (Carder and Steward, 1985), the equation becomes:

$$R_L = .1076\, (b_w + b_c C' + b_m M)/(a_w + a_c C + a_y Y) \qquad (2)$$

All terms have units of m^{-1}. The a and b absorption and scattering coefficients all have their own characteristic variation with wavelength, and are multiplied by the appropriate concentrations of the different constituents. The a_C and b_C, and to some extent the associated b_m, will vary with phytoplankton species and physiological state, but mean "normal" spectral curves are usually assumed.

The total absorption is due to water (a_W), to phytoplankton (a_c), and to dissolved yellow organic matter, or gelbstoff (a_y). Water gives a constant Rayleigh scatter (b_w) to which is added scatter from the phytoplankton (b_w) and from other suspended material (b_m). Morel (1980 and 1988) has suggested a relation for these backscatter contributions in which the coefficient C' varies non-linearly with C, the chlorophyll concentration in mg m^{-3}.

We have also added a term to include solar-stimulated fluorescence centered at 685 nm, taking account of absorption of both the stimulating and the emitted radiation (Neville and Gower, 1977, Spitzer and Dirks, 1986). The amount of fluorescence will depend on the absorption in the water. Absorption of stimulating light by phytoplankton gathers the energy to be emitted at longer wavelengths; any other absorption reduces the emitted fluorescence. The total emission is

controlled by a single yield parameter which is not necessarily dependent on chlorophyll concentration or other water properties, but which is expected to vary with growth phase and physiological state of the plankton. Typical values are 0.3 to 1%.

The above equation, even with the fluorescence term added, is simple in form, but is limited in practice by uncertainties in the spectral values of the coefficients. The terms b_w, a_w and a_y are relatively well defined, though measurements of Y (absorption m^{-1} at 350 nm) are often lacking in practice. The spectral form of the term b_c may be taken from the spectral reflectance of phytoplankton deposited in sufficiently high concentration on filter papers (Mitchell and Kiefer, 1988). The spectral form of the inorganic component is usually taken as having a uniform power law, typically (wavelength)$^{-1}$, across the visible spectrum. The dominant effect of the phytoplankton is through the term a_c, which can be measured in concentrated in-vivo conditions, or (with suitable corrections) from measured transmission through phytoplankton deposited on a filter paper.

3.2.2 *Modelled reflectance spectra.* We use the specific absorption coefficient of *Trichodesmium* shown in Figure 3, and the corresponding curve for the diatom *Skeletonema*, (figure 4) also measured with the IOS spectrometer in a laboratory culture to compute reflectance spectra with the above model.

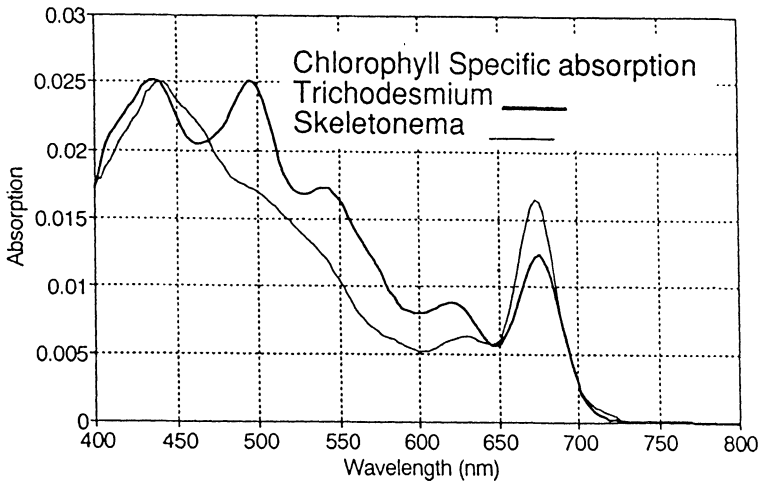

Figure 4. Normalized absorption coefficients for *Trichodesmium* and the diatom *Skeletonema* used in the reflectance modelling in figure 5 a to e.

Predicted pairs of reflectance spectra are shown in Figure 5a to 5f. The four pairs of spectra cover conditions ranging from relatively clear, oligotrophic water to fairly concentrated near-bloom conditions. In Figure 5a the two spectra in clear water (C = .1 mg m^{-3}, b = .1 m^{-1}, Y = .005 m^{-1}) are indistinguishable. In Figure 5b (C = 1 mg m^{-1}, b = .5 m^{-1}, Y = .01 m^{-1}) the differences start to become appreciable, and should be easily measurable with a sensor having sufficient spectral resolution in cases illustrated in Figures 5c (C = 10 mg m^{-1}, b = 2 m^{-1}, Y = .02 m^{-1}) and 5d (C = 25 mg m^{-1}, b = 2 m^{-1}, Y = .02 m^{-1}).

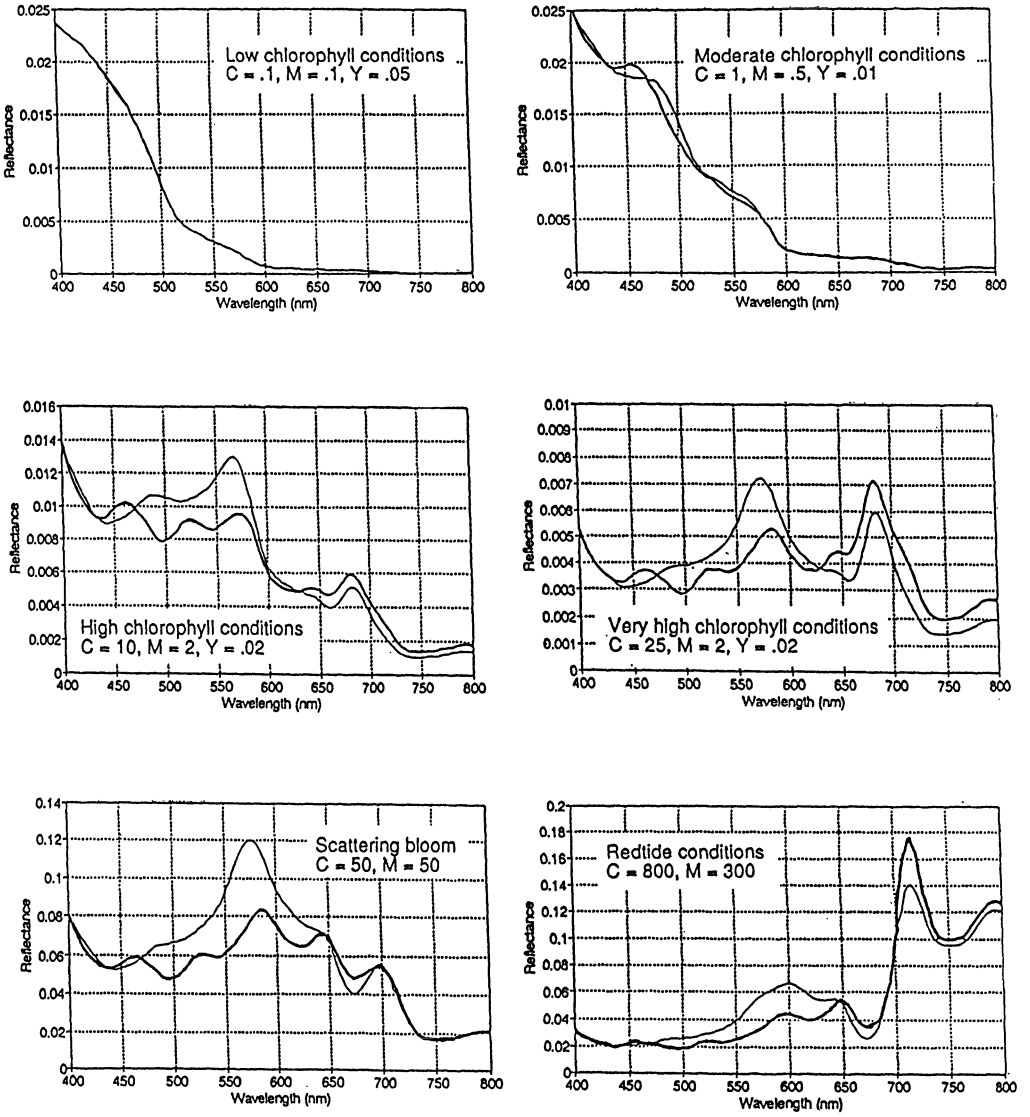
Low chlorophyll conditions
C = .1, M = .1, Y = .05
Moderate chlorophyll conditions
C = 1, M = .5, Y = .01
High chlorophyll conditions
C = 10, M = 2, Y = .02
Very high chlorophyll conditions
C = 25, M = 2, Y = .02
Scattering bloom
C = 50, M = 50
Redtide conditions
C = 800, M = 300
Reflectance
Wavelength (nm)

Figure 5a to e. Modelled reflectance spectra for *Trichodesmium* (heavy line) and *Skeletonema* (thin line) for varying concentrations of chlorophyll (C), scattering (M) and Gelbstoff (Y).

In each case in Figure 5 a constant fluorescence yield of 1% has been used. This fluorescence emission, centered on about 685 nm, overlaps the absorption band of chlorophyll a centered on 670 nm. Under low-scattering conditions the fluorescence peak provides a clear and useful signal for estimation of chlorophyll concentrations in the range 0.5 - 25 mg m^{-3} (Gower and Borstad, 1981).

We also show the results for more extreme conditions in Figure 5e (C = 50 mg m^{-1}, b = 50 m^{-1}, Y = .02 m^{-1}) where a bloom with high scattering is modelled, and 5f (C = 800 mg m^{-1}, b = 300 m^{-1}, Y = .02 m^{-1}), which was designed to duplicate the form of reflectance spectra observed in a red tide of the dinoflagellate *Gonyaulax spinifera* off the west coast of Vancouver Island in August and September 1990 (Gower and Borstad, 1991). In these two cases the fluorescence signal is lost in the larger effect of chlorophyll absorption. As the amount of scattering near 670 nm increases, the absorption will cause an increasing dip, which compensates for the fluorescence peak and then dominates the spectrum

3.3. MEASURED REFLECTANCE SPECTRA

3.3.1. *Reflectance Spectra of a Serial Dilution of Concentrated Trichodesmium.* Because a natural bloom was not encountered on the November 1988 Iselin cruise, we created bloom-like conditions by pouring the contents of a near-surface plankton tow into a small, clear plastic container suspended in the sea off the sunny side of the ship. While this artificial situation will not mimic the real optical behaviour of a *Trichodesmium* bloom, we feel that it a good first approximation since in a bloom the concentrations near the surface would be very high.

At one station off Roatan Island, (Honduras) the contents of a 64 μm mesh plankton tow were poured into the container, which was 10 cm diameter and 25 cm deep, with a rounded bottom which helped avoid reflection from the container itself. Upwelling radiance spectra were obtained by pointing the spectrometer vertically into the container from the deck of the ship. A file of 100 or more individual spectra were obtained for each dilution, from which averages of 10 to 20 spectra from inside the container were later selected to represent that dilution. After each raw radiance data file was obtained, the contents of the container was diluted with an equal volume of sea water, and another file of spectra were measured. After the first two spectra were obtained (0 and 1 in Figure 6), the plankton was gently screened through a 64 μm mesh plankton net in an attempt to wash out some of the contaminating diatoms and nannoplankton. Samples at each dilution were counted using a microscope and Sedgwick-Rafter cell, and chlorophyll and phycoerythrin concentrations were obtained for most dilutions.

The raw upwelling radiance spectra were transformed into reflectance factor spectra by dividing by a radiance spectrum of a white card obtained at the same time. A very small correction for the reflectance contribution of the container itself was calculated by viewing the container containing ambient seawater and subtracting the reflectance of the nearby sea itself. The corrected spectra obtained are shown in Figure 6.

3.3.2. *Above Water Reflectance of Trichodesmium.* Reflectance spectra were also measured on *Trichodesmium* samples concentrated on 2.54 cm GF/C filters by viewing them from above under solar illumination. In order to avoid further bleaching (since samples were collected from surface plankton tows, they perhaps already exhibited some bleaching), this measurement was made within a few seconds after the cells on the filters were first exposed to sunlight, though no evidence of bleaching was observed in subsequent measurements. The observed radiance spectra were normalized by the spectrum from a moistened, white filter viewed immediately afterwards in order to provide reflectance factor spectra.

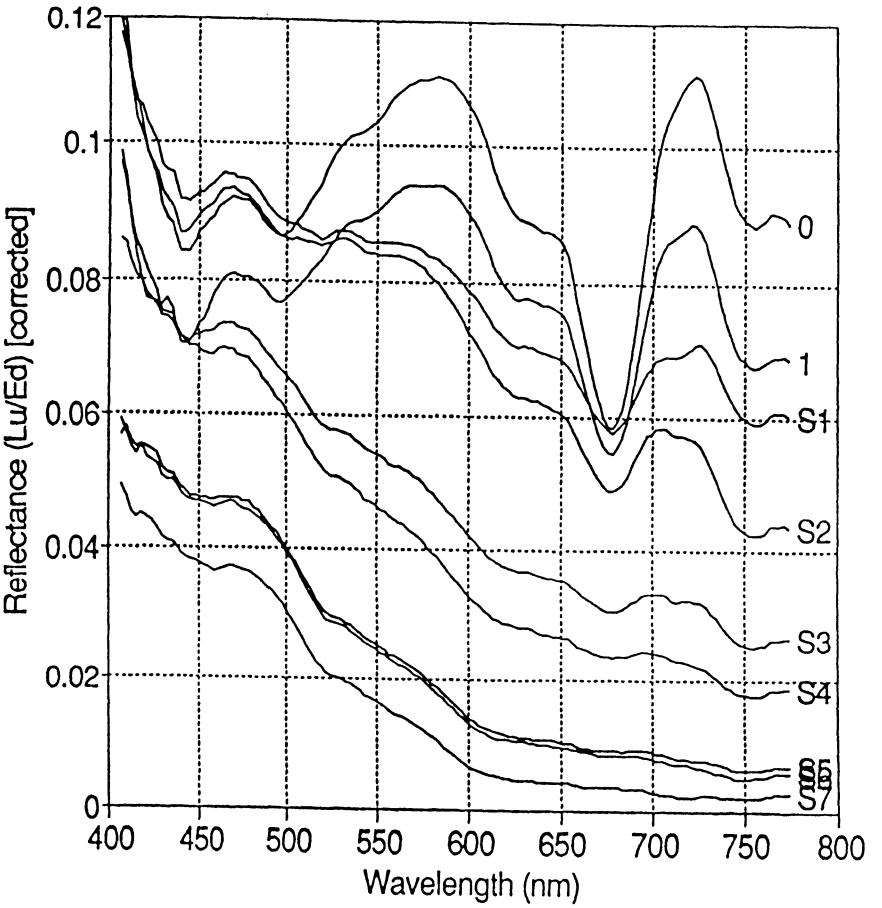

Figure 6. Spectral reflectance of a serial dilution of *Trichodesmium* measured in a small transparent container suspended at the surface of the sea in deep water. Dilutions 0 to S6 are 1441, 949, 858, 696, 359, 216, 144 and 46 trichomes ml^{-1}.

Results are shown in Figure 7 for concentrations of 1300 (upper curve), 2600, 5700, 6600, and 23000 (lowest curve) trichomes cm^{-2} referenced against a blank filter whose reflectance was assumed to be unity at all wavelengths. The centre curves show some variation in spectral form, but the samples show the expected trend from the thin covering of the filter at the lowest concentration to near optical thickness for the lowest curve. The reduction in reflectance of the filter for the numbers of trichomes cm^{-2} given above implies a cross-section area per trichome of 5×10^{-5} to $2 \times 10^{-4} cm^2$. This agrees very well with mean cell diameters of 10 μm and trichome lengths of 1 mm which gives an area of $10^{-4} cm^2$.

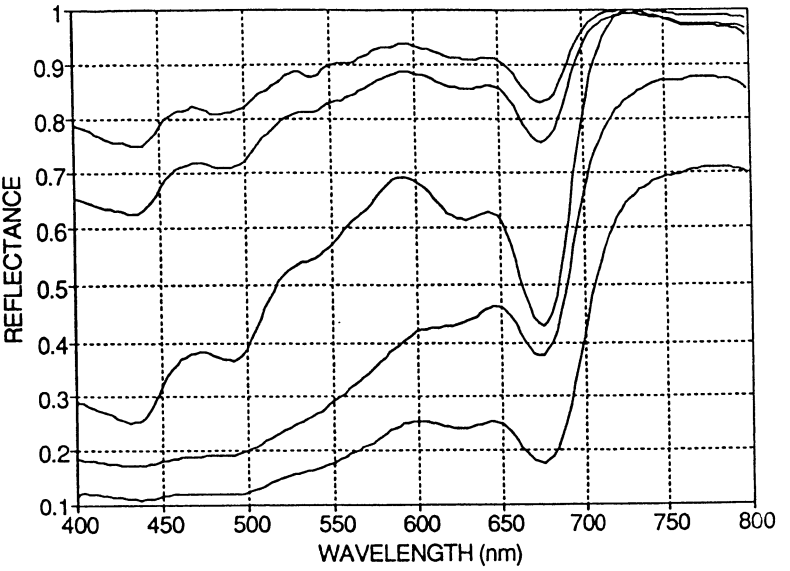

Figure 7. Reflectance of mats of *Trichodesmium* on white GF/C filter papers, to mimic surface scums of the alga without overlaying water.

4. Implications For Remote Sensing

4.1. SPECTRAL BAND REQUIREMENTS

The absorption spectrum in Figure 3 for *Trichodesmium* indicates sufficient similarity to other marine phytoplankton that measurements of "effective" pigment absorption using a ratio of blue and green upwelling radiances, for example with CZCS satellite data, will probably be satisfactory at "sub-bloom" conditions as suggested by Lewis et al 1988. However, the conversion of the "effective" pigment concentration to equivalent measurements in other species needs to take account of the self-shading noted above, since the colonial nature of *Trichodesmium* wm result in an underestimate of tropical and sub-tropical chlorophyll.

The three widely spaced CZCS spectral bands can not detect the spectral variations at 495 nm or the smaller variations at 545 nm due to the phycobilin pigments in cyanobacteria, and so can not begin to distinguish cases where these pigments occur. Higher spectral resolution imagers are required for this. The Seawifs scanner, due to be launched in 1995, will have additional bands which should make some distinction possible, but the model results in section 3 indicate that more bands are needed.

Minima in both the modelled and measured *Trichodesmium* reflectance spectra (figures 5 and 6 respectively), correspond to chlorophyll absorption at 440 and 670 nm, to phycoerythrin absorption at 495 and 545 nm and to phycocyanin absorption at 625 nm. In the red region of the spectrum of water-leaving radiance, we recognize solar-stimulated chlorophyll a fluorescence at 685 nm (Neville and Gower 1977, Gower and Borstad 1981, Borstad et al., 1985). In general at least two bands are needed (one in the absorption or fluorescence region and at least one other close by as a reference) to confirm the presence of such features, implying the need for 10 bands

in the spectral region 440 to 670 alone, with additional bands for atmospheric correction, gelbstoff determination and measurement of fluorescence. In combination with the need to make remote sensing observations in narrow and well-defined windows that minimize contamination by atmospheric absorption features, we are left with a requirement for nearly continuous coverage of the visible spectrum, suggesting that imaging spectrometers such as the Fluorescence Line Imager (Borstad et al, 1985) and the Compact Airborne Spectrographic Imager (Borstad and Hill, 1989) may be the appropriate technology.

The model spectra also indicate that measurements of solar-stimulated fluorescence should be a useful capability in a space sensor. In micro-photometric studies of *Trichodesmium* absorption and fluorescence Carpenter (unpublished) has recorded fluorescence at 560 - 575 nm (from phycoerythrin), 655 nm (from Phycocyanin) and 681 nm (from chlorophyll) with 490 nm stimulation. Relative to our modelled reflectance, and to reflectance of natural blooms of diatoms or dinoflagellates measured with the same instrument, our measured *Trichodesmium* reflectance spectra show a shoulder at 655 nm. We have seen this shoulder in remote sensing measurements of other blue-greens in freshwater lakes also, but it is not commonly seen in diatom or dinoflagellate blooms. This may be evidence of solar stimulated phycocyanin fluorescence, however, without detailed measurements we can not separate this feature from the effects of absorption occurring in the same region. We do not recognize fluorescence by phycoerythrin, presumably because it is included in the main green peak at 575 nm. Hoge and Swift (1983) have shown that it is possible to remotely measure phycoerythrin fluorescence using active laser stimulation from aircraft. Specific identification of *Trichodesmium* may have to rely on actively stimulated fluorescence of phycoerythrin (Hoge and Swift 1983), or on its absorption properties. Methods based on phycoerythrin fluorescence may be confounded by interference from *Synechococcus*, another abundant cyanobacterium.

4.2. RED REFLECTANCE OF AN ALGAL MAT OR SURFACE SCUM

The reflectance spectra in Figure 7 show the effect of increasing coverage by trichomes on the filters, with the spectra for the highest concentrations showing the "red edge" at 720 nm characteristic of terrestrial vegetation. The reflectance of the more concentrated samples are also similar to the upwelling radiance spectra of benthic cyanophyte mats shown by Jorgensen and De Marais (1988). Surface mats or scums of *Trichodesmium* and other blue-greens will show the characteristic chlorophyll "red edge" in their spectrum, a feature which should be detectable in remote sensing measurements over deep water by the radiance difference between 680 and 720 nm.

All chlorophyll containing plant species show this "red edge" when viewed directly with no water cover. This feature is a function of the low absorption of chlorophyll pigments at wavelengths longer than 700 nm. When dispersed in low concentrations in water, the increasing water absorption reduces the reflectance at wavelengths longer than 720 nm, leading to formation of a peak at wavelengths between 710 and 730 nm.

The effect of this "red edge" can be seen in images of surface mats of other bloom forming species acquired with the Advanced Very High Resolution Radiometer (AVHRR) on board the American NOAA weather satellites (Gower and Borstad, 1991). In extensive surface scums the ratio of radiance increases in the infrared to that in the red, above the radiances observed in nearby clear water, are close to the value for vegetation on land. The actual increases allow one to calculate the fraction of the sea surface within the sensor field of view which is covered with such vegetation. Similar measurements of *Trichodesmium* should be possible with the AVHRR where a sufficient fraction of the 1 km^2 pixel field of view of the scanner is covered. The AVHRR has the daily coverage needed to follow such blooms, and provides measurements in the

visible and near infrared with sufficient sensitivity. However, its spatial resolution limits its usefulness to cases where large areas (greater than several square km) are affected.

4.3. VARIATIONS OF COLOR WITH BLOOM PHASE

Not all *Trichodesmium* blooms are the silver-yellow color we saw in our serial dilution experiment. Daniel et al (1976) describe the moderate (200 - 450 trichomes ml^{-1}) concentrations as feeble to uniform greenish yellow in color. Devassy et al., (1978) describe the early stages of a bloom off Goa in India as grey in color, then... "As the bloom gets older, it begins to impart shades of reddish brown color and in bright sunlight it makes the sea look reddish in wavy lines. After some days, the bloom gets more and more concentrated and gives distinct reddish brown coloration to the water". Creagh (1986) summarizes reports of the color of *Trichodesmium* blooms off Australia as "extremely dense yellow green and brown; pale brown; high density brown; and very dense milky blooms."

These color variations are due to changing algal concentrations, but they must also relate to changes in the intra-cellular pigment concentrations and to the degree of vacuolation, which in many blue-green alga is under physiological control. Unfortunately absorption or reflectance spectra of trichomes with deflated gas vesicles have yet not been obtained. Walsby (1978) has reported a decrease in turbidity of about 35% using a nepholometer when *Trichodesmium* gas vesicles are collapsed using pressure. We did not measure scattering in our serial dilution experiment, but the model can be manipulated to show the effects of changes in scattering. The shape of the curves in Figure 6 and the increase in signal levels at all wavelengths with increasing *Trichodesmium* concentration indicates scattering over the full range of wavelengths plotted in our artificial "bloom". As expected, there was a very high correlation between reflectance at 780 nm and trichome concentration in our samples (R_{780} = 6.2 x 10^{-5} trichomes ml^{-1} +.0068; r^2= .98).

The variation in bloom color will require different algorithms for different stages of a bloom. However, high reflectance at wavelengths greater than about 710 nm will allow surface scums of *Trichodesmium* and other algae to be visible in AVHRR and Landsat imagery regardless of the stage of the bloom. It should be noted however this scattering would be misinterpreted by the standard CZCS processing algorithm, which assumes zero water-leaving radiance at wavelengths longer than 670 nm. Higher spectral resolution and sensitivity with future sensors should give greater sensitivity, and permit positive identification of pigments such as phycoerythrin and phycocyanin.

5. Concluding Remarks

We have demonstrated that even with the cursory knowledge of spectral absorption and reflectance properties of *Trichodesmium* we have at present, it should be possible to design remote sensing algorithms specific to this cyanobacterium, especially when high spectral resolution devices are available. At present, some useful work can be done with data from conventional satellite sensors having broad spectral bands. Under conditions of intense blooms exhibiting surface scums, the long wavelength reflectance should provide a quantitative measure of abundance. Large area blooms can be mapped with the AVHRR, and smaller area events can sometimes be mapped with Landsat imagery. At very low concentrations, the conventional blue-green methods (Gordon et al, 1983) should suffice and CZCS data should be useful. However, algorithms will have to be altered to take into account the packaging or self shading occurring in colonies. At concentrations between about 5 and 200 mg, high spectral resolution

imaging spectrometers will provide the ability to detect and measure absorption from accessory pigments.

At present there are no satellite sensors in orbit designed for measuring ocean color. With the launch of Seawifs in 1995, the capabilities of satellite sensors will finally surpass those of the CZCS which provided data over the period 1978 to 1986. Seawifs will not however have the spectral bands needed to make a full study of the spectral information that should be available to satellite sensors. For this imaging spectrometers are required. Such instruments are now available on aircraft (AVIRIS, CASI, FLI, ROSIS) and will be available in space starting with the MERIS on the European EOS system in about 1998.

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NITROGEN FIXATION IN *TRICHODESMIUM* BLOOMS

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ABSTRACT. Blooms of *Trichodesmium* are common in tropical and subtropical seas of all oceans, particularly when winds are calm and water temperature exceeds 26°C. If as few as twenty moderate-size (20,000 km^2) blooms occur globally and last for 15 days per year, a total of 5.4 Tg N would be fixed, a value which would more than double the current estimate of pelagic N_2 fixation by *Trichodesmium* in the world's oceans.

1. Introduction

In estimating global rates of nitrogen fixation in the pelagic zone of the sea, Carpenter (1983) and Capone and Carpenter (1982) calculated the rate of nitrogen fixation by the planktonic cyanobacterium *Trichodesmium* to be 4.7 Tg yr^{-1}. Because so little information existed on blooms, this value was based only on non-bloom concentrations and rates of N_2 fixation by *Trichodesmium*. In the past decade, more information has accumulated on blooms, and we present here a global estimate of N_2 fixation in these events. Precise estimates of bloom size and frequency, however await the establishment of a new generation of remote sensors which can distinguish *Trichodesmium* from other phytoplankters. Development of algorithms for detecting *Trichodesmium* appears possible because of the highly reflective nature of gas vesicles in *Trichodesmium* and the presence of photosynthetic accessory pigments such as phycoerythrin.

2. Historical Observations On Blooms

A bloom, or a noticeably high concentration of *Trichodesmium* on the sea surface, may be as small as a windrow or may cover tens of thousands of km^2. One of the earliest records of a bloom was one observed by E. Dupont (cited by Wille, 1904) in the Red Sea. Based on the ship speed, the length was about 256 nautical miles. The largest bloom yet recorded was detected by Dupouy et al. (1988) using CZCS satellite imagery. It occurred in New Caledonia and the

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Vanuatu archipelago in the South Pacific, and was estimated to cover 90,000 km^{-2}. Another extensive bloom was noted by Wood (1965) while flying over the Great Barrier Reef. He estimated the size was 52,000 km^2. A *Trichodesmium* bloom was observed by Karl et al. (1992) in August 1989 about 100 km from the Hawaiian archipelago. They calculated that N_2 fixation in blooms can supply up to 50% of the new nitrogen to the oligotrophic North Pacific Ocean. Numerous other reports exist in the literature, and Table 1 is a summary of information gathered on them.

There are several regions of the world which are particularly noted for high *Trichodesmium* bloom activity, and all of these are in tropical or subtropical regions. These are: shelf waters of north and northwest Australia (Hallegraeff and Jeffrey 1984, Creagh 1985), and Great Barrier Reef (Revelante and Gilmartin 1982, Jones et al. 1986), west (Devassy et al. 1978) and east (Madhupratap et al. 1980) coasts of India and east coast of Africa (Bryceson 1982) and Madagascar, (Sournia 1968) during the northeast Monsoon. Some other less known but important areas are the Gulf of Thailand (Suvapepun 1991) and the southwestern South Pacific ocean (Bowman and Lancaster 1965, Dupouy et al. 1988) Caribbean Sea, and Gulf of Mexico (Carpenter 1983).

3. Bloom Area, Depth , Duration, And Trichome Concentration

Blooms along the west coast of India occur between February and May during the Northeast Monsoon when waters are typically calm and warm (ca, 27-32° C). During this time, rate of photosynthesis reaches 500 mg C m^{-3} hr^{-1} (Devassy et al. 1978). The density of *Trichodesmium* is often extremely high, with trichome concentrations in mature blooms up to 38.6 x 10^3 ml^{-1}. These very high trichome concentrations along the west coast are sometimes only found in a relatively thin (ca. 5 cm) layer at the surface. However, other *Trichodesmium* blooms in this region have been reported to extend much deeper. For example, Quasim (1972) noted densities of *Trichodesmium* colonies at surface, 5 m, and 10 m to be 8.0, 7.0 and 5.0 $liter^{-1}$ in a bloom. At the surface at one station, Quasim (1972) observed 500 colonies $liter^{-1}$. These blooms along the west coast of India cover several hundred square nautical miles and appear intermittently every year through March to May. Blooms are typically several km long, parallel the coast and last for a month to several months (Devassy et al. 1978). Devassy (pers. comm.) for example, observed one bloom which was scattered along the coast from 13° 30'N to 15° 15'N (ca 105 nautical miles long) in March 1981 and another which was uninterrupted for 600 km between 15° N and 10° N in April 1984.

In the Gulf of Thailand (Suvapepun, this volume) concentrations of *Trichodesmium* are typically about 175 to 300 trichomes ml^{-1} extending through the upper 5m. A total of 51 blooms were observed between 1981 and 1990 in this area.

4. Rates Of N_2 Fixation

Nitrogen fixation rates have only been measured in a few blooms. Off Dar es Salaam, Tanzania, Bryceson (1977) observed rates of 18 n moles N per 1000 trichomes h^{-1}. (or 252 pg N $trichome^{-1}$ h^{-1}). Similarly, Saino and Hattori (1979) measured about 87 pg N fixed $trichome^{-1}$ h^{-1} in a bloom of *T. erythraeum* in the Kuroshio Current. On 11 November 1985, Capone (pers. obs.)

Table 1. Summary of data on blooms reported in the literature.

Author	Month	Location	Temp (°C)	Trichomes (ml^{-1})	Comments
INDIA					
Devassy et al.1978	Feb-Apr	W. Coast India	27-32	ca 1-39 x 10^3	calm weather
Daniel et al. 1977	mid-Apr	Madras	28.2	300-450	no rain or wind
Joseph 1982	Feb-Apr	Vellar estuary	28-29	210-320	
Devassy 1987	Feb-May	Goa	27-32	10-38.5 x 10^3	calm & sunny
Quasim 1972	April	Laccadive Isl.	29.7-30.6	1.3-125	
Madhupratap et al. 1980	N.A.	W. Bay of Bengal	27-29	124	bloom
AUSTRALIA					
Revelante & Gilmartin 1982	Aug-Jan	Great Barrier Reef	20-22	50 in bloom	blooms if wind < 5 m sec^{-1}
Jones et al. 1986	June	Great Barrier Reef	N.A.	5-12	maximum in June
Creagh 1985	Nov-Apr	Dampier Isl.	N.A.	725-9720 x 10^3	
Hallegraeff & Jeffrey 1984	All Year	N & W coast	N.A.	1 (max.)	All Year
Wood 1965	spring	Great Barrier Reef	N.A.	N.A.	bloom
SOUTH ATLANTIC					
Sato 1966	Oct	Recife	29.6	ca. 25.8 max	red tides
NORTH ATLANTIC					
Dunstan & Hosford 1977	July	Georgia Bight	27-29	1.87 (mean)	Trico was 50% of POC in July
Eleuterius et al. 1981	August	Gulf of Mexico	29-30	100 (windrows)	
Lewis et al. 1988	August	35N,65W	28	N.A.	
GULF OF THAILAND					
Suvapepun 1982	Feb-Apr	Gulf of Thailand	30-31	N.A.	
Suvapepun (this vol.)		Gulf of Thailand	27-31	176-314 , as high as 5700	
PACIFIC OCEAN					
Baas Becking 1951	N.A.	Great Barrier Reef	N.A.	N.A.	40,000 km^2 bloom
Bowman & Lancaster 1965	January	Tonga Isl	26	N.A.	
Dupouy et al. 1988	January	New Caledonia	N.A.	N.A.	90,000 km^2
Karl et al. this volume	August	100 km N Hawaii	27	N.A.	1000 km^2
Saino & Hattori 1979	July	32N,134E	28	N.A.	

Note: where *Trichodesmium* density values were calculated from cell or colony units, it was assumed that there were 100 cells $trichome^{-1}$, 250 trichomes $colony^{-1}$. Unless original author stated otherwise.

encountered a *Trichodesmium* bloom off Pandora Reef, in northeastern Australia. He did not count the density of trichomes, but assayed the bloom on a volumetric basis and calculated 0.24 mg N fixed m^{-3} h^{-1}(assuming a 3:1 conversion ratio) in surface water incubated using the acetylene reduction assay in the light. In a *Trichodesmium* bloom in the Virgin Islands, West Indies, on 19 April 1990, Carpenter, Capone and O'Neil (unpubl. data) noted a mean N_2 fixation rate of 49.5 mg N fixed m^{-3} h^{-1} (assuming 3:1 conversion ratio) in surface water samples. Water temperature was 26.7°C, and mean concentration of cells of *Trichodesmium* in the upper 2 meters was 48.6 x 10^6 $liter^{-1}$ (or about 500 x 10^3 trichomes liter, giving a rate of N_2 fixation of about 99 pg N fixed $trichome^{-1}$ h^{-1}.

Thus we have an observed range in rates of N_2 fixation of from about 87 to 252 pg N $trichome^{-1}$ h^{-1} in *Trichodesmium* blooms. The mean PN content of a colony of about 200 trichomes is approximately 2 ug N (Carpenter 1983), and if we assume a rate of N_2 fixation of 150 pg N $trichome^{-1}$ h^{-1} for a 12 h day^{-1}(150 pg x 12 h x 200 trichomes), we obtain 5.5 days PN turnover.

5. Global Rates Of N_2 Fixation In Blooms

Taking these past observations of blooms into consideration, we can make an approximation of the density and N_2 fixation rate in a "typical" bloom. Let us assume a rate of N_2 fixation of 150 pg N $trichome^{-1}$ h^{-1}, a *Trichodesmium* concentration of 100 trichomes ml^{-1}, which is a usual bloom density (Table 1), and 12 hr of N_2 fixation per day. If the bloom extended to 5 m in depth, the rate of N_2 fixed would be 0.9 g N m^{-2} day^{-1}. Blooms along the west coast of India are reported as being often about 600 naut mi long and 5 naut mi wide, and thus cover approximately 10,000 km^2. In such a bloom, there would be, over a 30 day period, a total of 2.7 x 10^{11} g N fixed. It should be noted that this is not a particularly large bloom, as it is only about a ninth the area of that observed by Dupouy et al. (1988).

The global frequency of occurrence of such blooms is not known. However, based on the observations presented in Table 1, it would not appear unreasonable to assume that globally twenty blooms of moderate size (20,000 km^2) or perhaps a few of the size observed by Dupuoy et al. (1988) might occur per year. If twenty of the smaller (20,000 km^2, 5 m deep) blooms occurred annually, each lasting only 15 days, this would result in about 5.4 Tg (10^{12} g) of N fixed, more than doubling the current estimate of pelagic N_2 fixation of 4.7 Tg (Carpenter 1983, Capone and Carpenter 1982).

6. Other Sources Of Pelagic N_2 Fixation

It is becoming apparent that some cyanobacterial diazotrophs, such as the endosymbiont *Richelia intracellularis*, are more abundant than previously assumed. This cyanobacterium is often observed in the diatoms *Rhizosolenia* and *Hemiaulus*. Villareal (in press) calculates that at typical (non bloom) abundances (100 cells $liter^{-1}$) of the diatom *Hemiaulus* in the North Pacific Gyre, this endosymbiont fixes almost 70 ug N m^{-2} day^{-1}. Bloom concentrations of 25,000 *Hemiaulus* cells $liter^{-1}$ have been reported in the North Pacific Gyre (Venrick 1974). Thus it is possible that rates of N_2 fixation by *Richelia* are considerably higher than previously calculated.

7. General Observations On Trichodesmium Blooms

Several general observations are apparent from our summary of the *Trichodesmium* bloom literature. These are:

1) Blooms may be either oceanic or coastal in their occurrence. For example, they may occur far from land (Karl et al. 1992), or parallel the shoreline in shallow water, often washing up on beaches (Creagh 1985).

2) Most blooms occur when surface water temperature is 27°C or warmer. The only documented bloom, that we are aware of, which occurred in relatively cool water is that noted by Revelante and Gilmartin (1982). This took place at a water temperature of 20-22°C on the Great Barrier Reef.

3) Measurements of rates of N_2 or C fixation during blooms indicate that the colonies are active, with turnover times of PN or PC of about 3 to 6 days.

Certainly, at some latter stage in the history of a bloom cells become inactive, however measurements do indicate that at least in the initial stages of a bloom colonies are active.

4) In some regions, such as the Indian Ocean, blooms are seasonal in their occurrence. For example, they only take place in this area during the NE Monsoon (November to April) when windspeed is low. In contrast, blooms on the Great Barrier Reef appear to occur at various times throughout the whole year.

In summary, simple calculations and assumptions based on previous measurements of *Trichodesmium* bloom area, frequency of occurrence, colony density and N_2 fixation rate indicate that the global rate of N_2 fixation by this organism is probably twice the previous estimate. Further refinement awaits the use of remote sensing instruments to quantify blooms.

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TRICHODESMIUM BLOOMS AND NEW NITROGEN IN THE NORTH PACIFIC GYRE

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ABSTRACT. *Trichodesmium* blooms are ubiquitous phenomena in tropical and subtropical oceanic waters. Due to their diazotrophic capability, these blooms may play a quantitative role in global new production. However, to date it has been difficult to quantify the importance of diazotrophy due to a combination of undersampling of natural populations and a lack of pure culture physiological studies. In August 1989, we encountered a $>1 \times 10^3$ km^2 *Trichodesmium* bloom in the North Pacific Ocean near the JGOFS Hawaii Ocean Time-Series (HOT) Station ALOHA. Analyses of particulate matter collected from the densest portion of the bloom showed enrichments of 3,375 to 7,787-fold for carbon (PC), nitrogen (PN), adenosine triphosphate (ATP) and pigments (chl *a* and phaeo) relative to typical non-bloom concentrations; phosphorus (PP) was enriched only 583-fold. Dissolved nitrogen pools (NH_4, $[NO_3+NO_2]$, DON) were also enriched within the bloom, and in the case of NH_4 by 27-fold. Budget estimates based upon seasonally-averaged N imports to and exports from the epipelagic zone at Station ALOHA suggest that diazotrophic cyanobacterial blooms may be an important source of new nitrogen to the ecosystem. Two models are presented and discussed in order to account for the initiation of these open ocean blooms, and to explain the uncoupling of N and P concentrations and the selective enrichments of P which are required to favor the growth of diazotrophic microorganisms.

1. Introduction

The North Pacific sub-tropical gyre is the largest uniform body of water on our planet and occupies approximately 40,000 km^2. These central gyre waters are characterized by relatively high and nearly uniform surface temperatures (23-28° C), a broad thermocline and a seasonally-variable mixed layer (40-120 m). The chemical/biological composition of the water column includes low nutrient concentrations in the euphotic zone with concomitant low planktonic biomass, low rates of primary production and low rates of particle flux. Despite these oligotrophic conditions, oceanic habitats are responsible for the majority of global ocean primary production (>80% of total), attributable to the large size of the open ocean province (Martin et al. (1987)).

In addition to their contribution to total primary production, the central ocean gyres are important contributors to the export of carbon (and associated elements) from the euphotic zone and, therefore, have a prominent role in global models of carbon flux. New production, as

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defined by Dugdale and Goering (1967), is generally believed to be a function of NO_3 flux into the euphotic zone, which in turn is controlled by diffusion, turbulent advection across isopycnal surfaces and, to a lesser extent, by atmospheric deposition. Nitrogen fixation in the euphotic zone, in theory, could also contribute to new production but, at the present time, this process has not been included in ecosystem models (Legendre and Gosselin (1989)). Although the occurrence of microbiological N_2-fixation in stratified, low-nutrient oceanic habitats is well-documented (Dugdale et al. (1961), Dugdale et al. (1964), Bowman and Lancaster (1965), Goering et al. (1966), Carpenter (1973), Mague et al. (1974, 1977), Carpenter (1983)), it has been difficult to obtain reliable estimates of the quantitative importance of this process due to an undersampling of open ocean environments (Carpenter and Price (1977), Howarth et al. (1988), Capone and Carpenter (1982)).

In October 1988, the Hawaii Ocean Time-Series (HOT) program established a deep-water time-series station in the oligotrophic North Pacific Ocean as part of the international Joint Global Ocean Flux Study (JGOFS). Since that time, a suite of hydrographic and biogeochemical data have been collected, at approximately monthly intervals, to assess oceanic variability in order to provide a more comprehensive understanding of upper ocean processes. During August 1989, an extensive bloom of the phototrophic, N_2-fixing cyanobacterium *Trichodesmium* sp. was encountered near our study area (Figure 1). This paper: (1) presents data on the dissolved and particulate chemical characteristics observed during this bloom, (2) describes the potential importance of these bloom phenomena in new production estimates of the oligotrophic North Pacific gyre, and (3) presents two models to explain the initiation and maintenance of these blooms based upon allochthonous inputs of phosphorus.

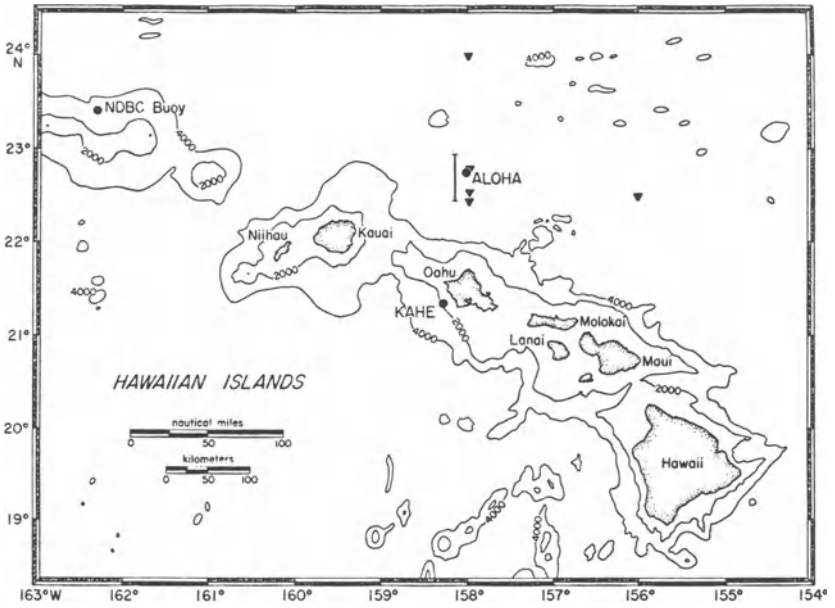

Figure 1. Map of study area showing the locations of Station ALOHA, NDBC Buoy #51001 and the region of the August 1989 *Trichodesmium* bloom. The solid triangles indicate locations where a dense surface accumulation of colonies was observed by aircraft and the solid line indicates the region of shipboard observations. A surface manifestation of *Trichodesmium* was not apparent at Station ALOHA during this period.

2. Materials and Methods

2.1. BLOOM ENCOUNTER

Station ALOHA (A Long-term Oligotrophic Habitat Assessment) is located approximately 100 km due north of Oahu, Hawaii at 22°45'N, 158°W (Figure 1). The station is in deep water (4750 m), is at least 50 km upwind of steep topography associated with the Hawaiian Ridge and is free of biogeochemical influences of the Hawaiian Islands. For these reasons, we consider Station ALOHA to be a valid benchmark for the study of oligotrophic oceanic processes. Approximately monthly cruises to Station ALOHA support detailed measurements and experiments on ocean physics, chemistry and biology (Chiswell et al. (1990)). The sampling strategies, analytical protocols and quality control/quality assurance procedures are described elsewhere (Karl et al. (1990)).

During the HOT-9 cruise (22-27 August 1989) aboard the SSP *Kaimalino*, we encountered an extensive surface water accumulation of *Trichodesmium* near 22°48'N, 158°11'W. Although most of the organisms were present in aggregates floating at or near the surface, colonies could also be seen to depths of ≥ 5 m. The surface aggregates were golden brown in coloration and were spatially heterogeneous, being arranged in discrete bands or windrows (Figure 2). The latter could be the result of Langmuir circulation cells (Bainbridge (1957)) or perhaps other physical forces.

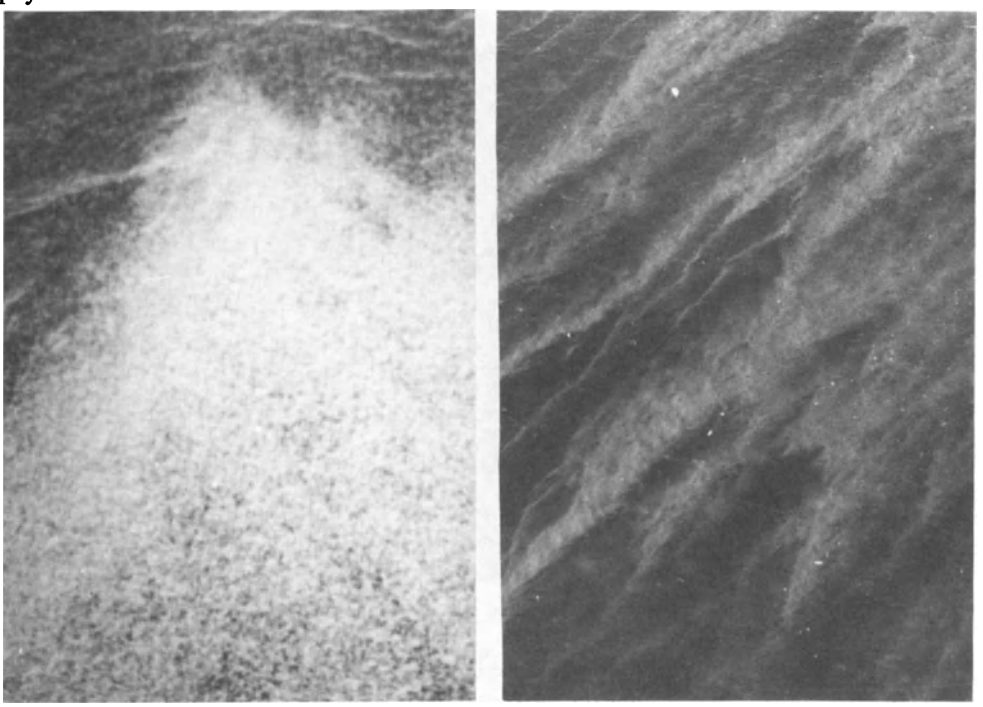

Figure 2. Photographic documentation of the *Trichodesmium* bloom showing the massive surface water accumulations of colonies: [A] shipboard view of the bloom from the *SSP Kaimalino* (22°48'N, 158°11'W), [B] aerial view of the bloom from the USCG C-130 aircraft (24°02'N, 158°W, altitude = 1,230 m). Photo credits: [A] D. Hebel, [B] K. Louder.

Trichodesmium blooms, defined here as surface water accumulations of viable colonies, are clearly dependent upon stability of the water column. It has been suggested that climatic conditions of low winds and calm seas favor bloom formation (Carpenter and Price (1976)). For the August 1989 bloom near Station ALOHA, local weather conditions were consistent with these prerequisites (Figure 3). For approximately 5-7 days antecedent to our encountering this bloom, the NDBC buoy #51001 registered decreasing winds and decreasing wave height (i.e., surface turbulence).

Independent of our research cruise and field observations, the United States Coast Guard (USCG) also encountered this plankton bloom during a C-130 aerial survey of Hawaiian waters in response to reports by local fisherman of "pollution." From their photographic logs recorded on 26 August 1989, we are certain that the ocean surface manifestation of this *Trichodesmium* bloom intermittently covered an area at least 1 x 10^3 km^2 (Figure 1). The bloom appeared to be truly oceanic and was not contiguous with the shallow portions of the Hawaiian Ridge.

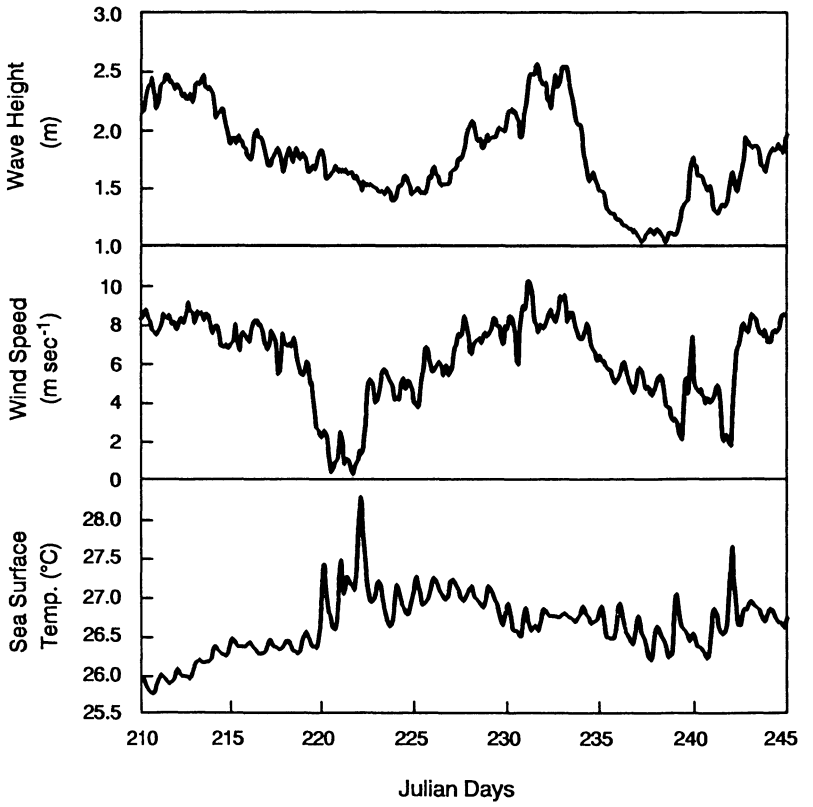

Figure 3. Time-series records of wave height, wind speed and sea surface temperature at NDBC buoy #51001 (see Figure 1) for the period 29 July - 3 September 1989. The bloom was sampled on Julian day #238.

It is important to mention that we are uncertain whether the observed bloom was due to a physical concentration of cells which were previously distributed over a larger horizontal or vertical region, or whether the bloom was the result of net growth of a "seed" population of *Trichodesmium* in the surface waters (Borstad (1982)). This is obviously more than a semantic distinction. Data presented below will demonstrate that the colonies sampled near the sea surface are viable and growing. In either case, the presence of a dense biomass of *Trichodesmium* colonies will have an impact on bioelement distributions and cycling rates in the oligotrophic North Pacific Ocean.

2.2. SAMPLING AND ANALYSES

Due to the constraints of ship time and the serendipitous nature of this discovery, no comprehensive sampling or experimental program was conducted. Nevertheless, surface waters were deliberately collected from "high" and "low" cell density portions of the bloom. Samples were collected using a tethered, plastic bucket, and were mixed thoroughly before subsampling. Portions were removed and processed for: (1) direct microscopic analyses, (2) particulate carbon, nitrogen and phosphorus (PC, PN, PP), (3) particulate adenosine triphosphate (P-ATP), (4) dissolved inorganic nutrients (PO_4, $[NO_3+NO_2]$, SiO_2), (5) dissolved organic nutrients (DOC, DON, DOP), (6) pigments and (7) primary production using the standard HOT program procedures (Karl et al. (1990)). Meteorological data including: air temperature (in °C, to nearest 0.1), wind speed as 8.5 minutes average (in m sec^{-1}, to nearest 0.01), significant wave height corrected for low frequency noise (in m, to nearest 0.1) and sea surface temperature (in °C, to nearest 0.01) were obtained from the NOAA-National Data Buoy Center (NDBC) from ocean buoy #51001 located at 23°24'N, 162°18'W (Figure 1).

3. Results

Compared to the pre-bloom (July 1989) environmental conditions at Station ALOHA, surface ocean waters in the densest portions of the bloom were enriched 583 to 7,787-fold for a variety of suspended particulate matter parameters (Table 1). However, compared to the concentrations of PC, PN, chl *a* and ATP, suspended PP concentrations were relatively low. The bulk C:N:P molar ratio of particulate matter within the bloom was 891:125:1 compared to the more typical, pre-bloom elemental ratio of 142:20:1; however, the C:N ratios were identical for the two data sets. These elevated C:P and N:P molar ratios observed during the bloom are a strong indication of a P-limited population (Sakshaug and Holm-Hansen (1977)). Compared to chemical determinations obtained for suspended matter samples of previously studied *Trichodesmium* blooms, our samples had similar PC:PN, PC:ATP, [ATPx250]:PN, PC:chl *a* and PC assimilation ratios (Table 2). Alkaline phosphatase activity (Karl and Craven (1980)) was readily detectable in extracts of the *Trichodesmium* colonies.

Water samples collected from the *Trichodesmium* bloom and analyzed for dissolved inorganic and organic nutrients had elevated concentrations of dissolved nitrogenous compounds including $[NO_3+NO_2]$, NH_4 and DON relative to the pre-bloom conditions (Table 3). Enrichments ranged from 3.1-fold for DON to >27-fold for NH_4 (Table 3). By comparison, neither dissolved P nor SiO_2 concentrations displayed any significant enrichments over the July 1989 levels. A comparison of dissolved nutrient concentrations sampled from within a dense patch of *Trichodesmium* colonies to a second sample collected near, but outside of the accumulation of cells also revealed that N compounds (but not P compounds) were enriched within the colonies (Table 4). Clearly the *Trichodesmium* colony consortium is a point source of dissolved N for the euphotic zone.

TABLE 1. Chemical composition of suspended particulate matter collected during the August 1989 *Trichodesmium* bloom

Parameter	Pre-Bloom[1]	Bloom	Enrichment Factor
PC (g m^{-3})	0.03	108 109	3,617
PN (g m^{-3})	0.005	19.3 16.9	3,620
PP (g m^{-3})	0.0006	0.36 0.34	583
chl *a* (mg m^{-3})	0.15	1168 (±400)	7,787
phaeo (mg m^{-3})	0.018	116 (±80)	6,444
ATP (mg m^{-3})	0.040 (±0.001)	135 (±12)	3,375
C:N:P molar ratio	142:20:1	891:125:1	
C:N molar ratio	7:1	7:1	

[1] data from surface waters collected at Station ALOHA in July 1989 approximately 1 month prior to the August 1989 *Trichodesmium* bloom sampling

TABLE 2. Comparison of the Station ALOHA bloom samples to other previously published studies

Parameter	McCarthy and Carpenter[1] (1979)	Mague et al. (1977)	Lewis et al. (1988)	This study
PC:PN (atoms)	6.1	4.7	7.0	7.1
PC:ATP (weight)	650	746	-	815
[Bio-C]:PN[2] (atoms)	2.4	1.4	-	2.2
PC:chl *a* (weight)	242	285	136	95
assimilation number (g C/g chl *a* · hr)	-	0.38	3.8[3]	0.17

[1] data for "puff" colony morphology
[2] "Bio-C" = ATP x 250
[3] based upon P_{max} estimates derived from single trichomes

TABLE 3. Dissolved nutrient concentrations (all in micromoles per liter) for water samples collected prior to and during the 1989 *Trichodesmium* bloom

Parameter	Pre-Bloom[1]	Bloom	Average Enrichment Factor
PO_4	0.10	0.13	1.3
DOP	0.22	0.23	1.0
Si	1.5	1.4	0.9
$[NO_3+NO_2]$	0.02	0.19	9.5
NH_4	<0.05	1.35	>27
DON	4.2	13	3.1

[1] data from surface waters collected at Station ALOHA in July 1989 approximately 1 month prior to the August 1989 *Trichodesmium* bloom

TABLE 4. Dissolved nutrient concentrations (all in micromoles per liter) associated with dense accumulations of *Trichodesmium* colonies

Parameter	outside of dense patch	within dense patch	Average within patch enrichment factor
PO_4	0.13 (±0.01)	0.13 0.14	1.0
DOP	0.19	0.22	1.2
$[NO_3+NO_2]$	0.08 (±0.012)	0.17 0.20	2.3
NH_4	0.23 (±0.35)	1.39 1.50	6.3
DON	5.0	10.5 10.6	2.1

At the time of sampling, the *Trichodesmium* colonies were positively buoyant. From direct microscopic analyses of samples collected from the densest portion of the bloom, we estimate a population of 2×10^5 *Trichodesmium* cells ml^{-1} (approximately 170 cells per trichome) in the surface waters. Based upon microscopic measurements of cell dimensions, we further estimate a *Trichodesmium* biovolume of 5.3×10^8 μm^3 ml^{-1}. From our ATP determinations (Table 1) and by assuming a C:ATP ratio of 250 (Karl (1980)), we estimate a biomass-C of approximately 34×10^{-6} g C ml^{-1} for the total microbial community. If we use these two independently-derived estimates of biovolume and biomass to calculate the C-specific bulk

density (i.e., mass [carbon] per unit volume), we derive a value of 6.4 x 10^{-2} g C cm^{-3}, which is within the range of previous estimates for the C content of a variety of microorganisms (reviewed in Karl (1986)).

Direct measurements of carbon production (using light and dark ^{14}C-HCO_3^- incubation methods) indicate that net photosynthesis (light-dark) for the *Trichodesmium* colonies sampled from the densest portion of the bloom was approximately 2.5 g C m^{-3} d^{-1}. At this rate, the estimated turnover time of the total PC pool during the *Trichodesmium* bloom would be 43 d, compared to a value of 7-10 d for surface water PC at Station ALOHA under non-bloom conditions.

4. Discussion

New production in oligotrophic oceanic environments is believed to be controlled by the upward transport of NO_3 from beneath the euphotic zone (Eppley and Peterson (1979)). Although allochthonous inputs of "new" nitrogen into the euphotic zone can occur by several alternative processes (i.e., atmospheric deposition, biological N_2 fixation, upward transport of particulate matter, animal migrations, etc.), cross-thermocline transport of NO_3^- is generally considered to be the dominant process. For the surface waters of the central north Pacific Ocean (Figure 4), the inventory of dissolved N is dominated by N_2 (400-500 μM) followed by DON (5-10 μM); [NO_3+NO_2] are barely detectable ($\leq$5 nM). Assuming that N is the limiting nutrient in oceanic habitats (Codispoti (1989)), there should be a strong selection for photosynthetic diazotrophs in low nutrient regions of the world ocean.

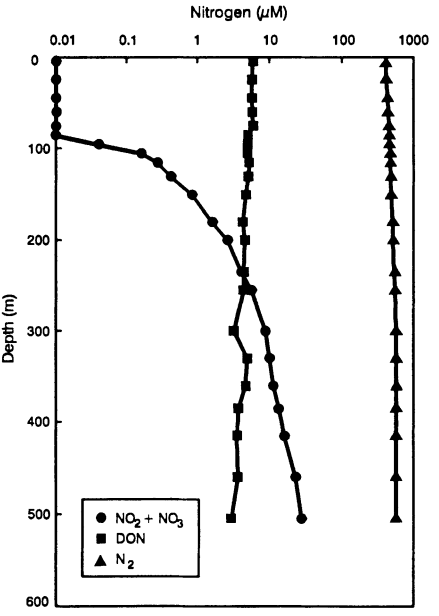

Figure 4. Average profiles of dissolved nitrogenous compound concentrations versus depth for samples collected at Station ALOHA showing the overwhelming importance of dissolved N_2 in the total N inventory. Note the logarithmic scale for concentration. In surface waters ($\leq$100 m) the [NO_3+NO_2] concentrations are typically $\leq$10 nM.

Blooms of the diazotrophic cyanobacterium, *Trichodesmium*, have been frequently reported from diverse oceanic regions, including: the North Atlantic Ocean (Lewis et al. (1988)), the tropical Atlantic Ocean (Darwin (1845), Goering et al. (1966)), the Sargasso Sea (Dugdale et al. (1961, 1964), Carpenter (1973), Carpenter and McCarthy (1975)), the Caribbean Sea (Carpenter and Price (1976), Li et al. (1980), Borstad (1982), Carpenter et al. (1987), Capone et al. (1990)), the western portion of the Gulf of Mexico (Hulburt (1962)), the North Pacific Ocean (Mague et al. (1974,1977), Saino and Hattori (1978)), the south Pacific Ocean (Bowman and Lancaster (1965), Dupouy et al. (1988)), the Red Sea (Ehrenberg (1830), Kimor and Golandsky (1977)), the Great Barrier Reef lagoon (Revelante and Gilmartin (1982)), and the Indian Ocean (Qasim (1970), Devassy et al. (1978), Bryceson and Fay (1981)). In spite of these relatively frequent bloom encounters, we are lacking a comprehensive and quantitative model to explain the processes of bloom formation, maintenance and eventual demise. In particular, the formation and maintenance of the blooms under environmental conditions of chronic nutrient limitation are especially intriguing and enigmatic.

We have recently assessed the potential contribution of N_2-fixation to total new production in the oligotrophic North Pacific Ocean (Table 5; Karl et al. (1991)). Our 1989 budget estimates,

TABLE 5. Annual N budget for US-JGOFS Station ALOHA for 1989 (from Karl et al. (1991), submitted for publication)

Rate Process	N Flux (mmol N m^{-2} yr^{-1})
Accumulations	
Gross Primary Production[1]	2326
Conventional Exports	
New Production[2]	140
New Production[3]	256
Particulate Export[4]	138
Migrant Zooplankton[5]	21-65
Conventional Imports	
Upward Eddy Diffusivity[6]	41-95
Upwelling[7]	4-9
Atmospheric Fallout[8]	8-26
Nonconventional Import	
N_2 Fixation[9]	49
N_2 Fixation[10]	80-100

[1] from direct measurements of 12 hr shipboard and *in situ* incubations with ^{14}C-HCO_3^- during monthly cruises to Station ALOHA (Chiswell et al. (1990), assuming a molar N:C ratio of 0.151 (Redfield et al. (1963))

[2] based on our 1989 annual estimate of gross primary production, assuming a f-ratio of 0.06 for oligotrophic ocean waters (Eppley and Peterson (1979))

[3] based on our 1989 annual estimate of gross primary production, assuming a f-ratio of 0.11 ± 0.14 which was empirically derived from ^{15}N uptake measurements in the NE Pacific Ocean over an 18-month field investigation (Knauer et al. (1990))

[4] from direct measurements of particulate N collected in free-drifting sediment traps at Station ALOHA from a reference depth of 150 m (Chiswell et al. (1990))

[5] computation of active transport of N from the euphotic zone derived from estimates of dissolved N excretion of migrant epi- and interzonal plankton and nekton for a station in the central N. Pacific gyre (Longhurst and Harrison (1988))

[6] calculated from the range of measured monthly ($d[NO_3 + NO_2]/dZ$) gradients across the nitricline at Station ALOHA during 1989 (0.03-0.07 mmol N m^{-4}; Chiswell et al. (1990)), assuming an eddy-diffusivity coefficient (K_v) of 3.7×10^{-5} m^2 sec^{-1} measured for the oligotrophic Atlantic Ocean (Lewis et al. (1986); Flux = $K_v(dN/d/Z)$

[7] calculated from the measured mean concentration of $[NO_3+NO_2]$ in the upper portion of the nitricline (1-2 mmol N m^{-3}; Chiswell et al. (1990), at Station ALOHA during 1989, assuming an upward vertical velocity (w) of 0.012 m d^{-1} as estimated for the Pacific Ocean (Munk (1966); Flux = w[N])

[8] based on measurements of wet and dry deposition in the central Pacific Ocean near Hawaii (Duce (1986))

[9] based on depth- and time-integrated estimates of N_2-fixation (acetylene reduction) in the euphotic zone during 6 cruises in 1972 to a site near Station ALOHA (Gundersen et al. (1976))

[10] based on N production estimates for August 1989 *Trichodesmium* bloom derived from ^{14}C assimilation rates and measured N:C molar ratio of 0.14, and assuming that the N-fixing cyanobacteria are uniformly distributed in the upper 2 m and are active at these measured rates for only 1 day per year

based upon measured and derived annual estimates of N imports to and exports from the epipelagic zone, suggest that cyanobacterial N_2-fixation may contribute significantly to new production in this subtropical, oligotrophic oceanic environment (Figure 5). An independent

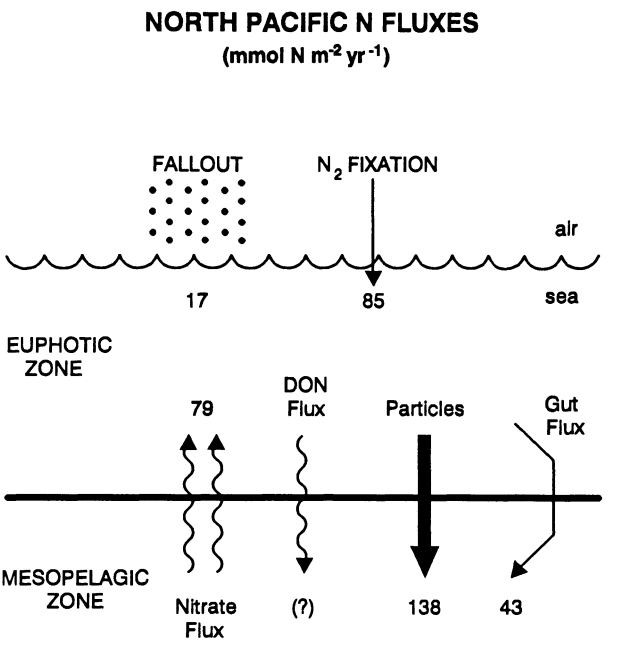

Figure 5. Schematic representation of approximate N-fluxes for the North Pacific Ocean from seasonally-averaged measurements and estimates (see Table 5 for more details).

study of the role of N_2-fixation in the tropical North Atlantic Ocean has arrived at a similar conclusion (Carpenter and Romans (1991)). If these results are characteristic of oligotrophic oceanic regions in general, Carpenter and Romans (1991) suggest a new "paradigm" for biological oceanography where the largest, rather than the smallest, phytoplankton play the major role in N-cycling and new production of the world ocean.

Although *Trichodesmium* can fix dinitrogen, and thereby relieve the ecosystem of "fixed" N-starvation, the absence of dissolved P (Doremus (1982)) and possibly other trace elements (e.g., Fe or Mo) which are also required for balanced growth of the plankton population will ultimately control the global importance of diazotrophy. Consequently, we submit that field studies of N_2-fixing cyanobacterial blooms should also include a careful and quantitative analysis of P-pool dynamics. An increased turnover rate of P, relative to N, is insufficient to satisfy the P demands during a bloom. There must be some specific mechanism responsible for spatially uncoupling P from N in the water column and for specifically concentrating P near the sea surface. However, in most oligotrophic oceanic regions the subeuphotic zone concentration versus depth profile for dissolved P is superimposed upon the dissolved N profile (generally with a concentration ratio of between 15-20 N : 1 P) such that neither upwelling, eddy-diffusion nor deep vertical mixing would act to selectively transport P into the surface ocean. Furthermore, the N_2-fixing plankton blooms are generally observed during periods of extreme calm (see Figure 3). Consequently, we can eliminate deep mixing as a mechanism for supplying P to surface blooms. Atmospheric deposition would likewise be ineffective at uncoupling N and P cycles or at selectively concentrating P because the N:P ratio of both wet

and dry deposition appears to be enriched in N (by 1-2 orders of magnitude) relative to cellular requirements (Duce (1986)).

The challenge, then, is to devise a model for the formation of N_2-fixing blooms that is consistent with both physiological and field data and that might serve as the basis for one or more testable hypotheses. We have developed two independent ecosystem models which may help contribute to bloom formation: (1) Upward P-Flux model and (2) *Trichodesmium* P-Transport model. These two models need not be mutually-exclusive.

In the Upward P-Flux model, phosphorus derived from the upward flux of low density organic matter, is taken up and assimilated by *Trichodesmium* cells that have independently accumulated at or near the ocean surface. The presence of alkaline phosphatase in the *Trichodesmium* colony consortia would allow the utilization of both organic-P and inorganic-P compounds. In support of this hypothesized upward flux model, several independent studies have made direct measurements of the upward vertical component of the flux of organic matter in the ocean (Simoneit et al. (1986), Smith et al. (1989)). For samples collected in the North Pacific gyre, the upward flux of organic matter (expressed in terms of carbon) was as high as 38% of the downward flux (Smith et al. (1989)). Although P was not measured in either of these studies, one might expect that the buoyant fraction of organic matter may be P-enriched (relative to N) due to the preponderance of phospholipids (Yayanos and Nevenzel (1978)). Although we do not routinely sample the surface microlayer of the ocean where the hypothesized enrichment of P would be most easily detected, we have observed surface mixed-layer enrichments of PO_4 at Station ALOHA (Karl and Tien (1991)), a feature which is consistent with our Upward P-Flux model.

However, this model is not without limitations. First, because the downward flux of P has no strong seasonality at Station ALOHA (Chiswell et al. (1990)), the hypothesized upward flux of P would be expected to be more or less constant throughout the year. Under these conditions, much of the P would probably be assimilated by organisms other than *Trichodesmium* simply due to the ephemeral nature of bloom occurrence. Finally, as stated previously, it is not certain whether the upward flux of organic matter can actually serve to spatially separate N and P in the oceanic environment. This would be a necessary prerequisite to select for diazotrophic cyanobacteria.

The *Trichodesmium* P-Transport model relies to a large extent on unique physiological attributes of *Trichodesmium* to establish the N-P separation and P concentration prerequisites (Figure 6). In this model, *Trichodesmium* cells are required to sustain a periodic transfer of P from depths of 125-150 m to the ocean's surface. This is accomplished by intracellular P storage at depth, perhaps as volutin granules (Harold (1966)), under light-limited, P-saturated conditions. The energy required to sustain the cells during this non-growing phase would be derived from excess carbohydrate previously stored under conditions of near surface light-saturation. Either an external stimulus (e.g., decreased turbulence) or an internal stimulus (e.g., exhaustion of stored carbohydrate, accumulation of P) triggers the *Trichodesmium* population to form gas vacuoles which allow the cells to change their position in the water column and, most importantly, increase the mean irradiance flux. Near the surface, these cells would be selected for due to their ability to fix N_2 and to maintain balanced growth at the expense of the stored polyphosphates. A surface "bloom" would result. When intracellular P is depleted, the cells continue to reduce carbon which is stored in the cell as carbohydrate. Dinitrogen fixation in excess of anabolic requirements may also continue under these conditions, which might account for the observed increase in dissolved N during the bloom (Tables 3 and 4). Because of the density of the storage carbohydrate, the cells become

negatively buoyant and sink to a position in the water column (perhaps determined by light, temperature or pressure) where active polyphosphate accumulation can again take place at the expense of carbohydrate catabolism. Given the published division rate estimates of natural populations (tens of days) and assuming the stored P can sustain 5-6 cell divisions, it is

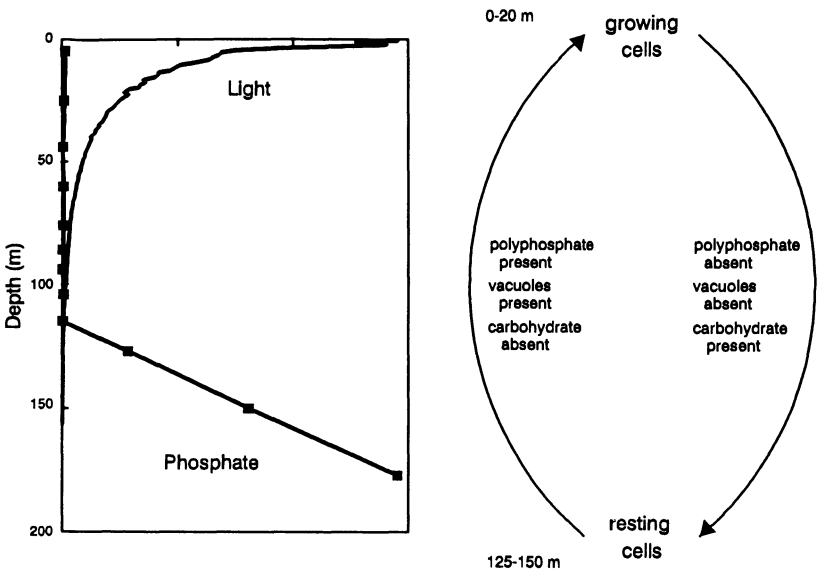

Figure 6. Schematic representation of the *Trichodesmium* P-transport model for the initiation and maintenance of surface ocean diazotrophic blooms. See text for more details.

conceivable that there exists a *Trichodesmium* cycle with a time scale of 100-150 days. This process of periodic migration is consistent with the 3-4 month "free-running cycle" of *Trichodesmium* that has been observed in the tropical Atlantic Ocean near Barbados (Steven and Glombitza (1972)).

In evaluating the potential validity of this model, it is important to note that buoyancy regulation by gas vaculation and the production of "ballast" carbohydrate in *Trichodesmium* is a well-documented phenomenon in cyanobacteria (Van Baalen and Brown (1969), Walsby (1978), Konopka (1984), Klemer (1985), Kromkamp and Walsby (1990)). In fact, buoyancy regulation in *Trichodesmium* must involve an active ballasting mechanism (Villareal and Carpenter (1990)) because turgor pressure collapse of the gas vesicles is not possible in this genus (Walsby (1978)). Furthermore, it is also known that the gas vacuoles of *Trichodesmium* are especially robust (Walsby (1978)), and can easily withstand the hypothesized 15-20 atm hydrostatic pressure required of this transport model. Also, it is well-known that the availability of dilute nutrients is enhanced by migration of the organisms (Munk and Riley (1952)), so movement even within the oligotrophic regions of the euphotic zone should confer a competitive advantage to *Trichodesmium* colonies for P assimilation. Although it is not known for certain whether *Trichodesmium* forms polyphosphate when supplied with excess phosphorus, this phenomenon has been documented in other related diazotrophic cyanobacteria (Stewart et al. (1978)).

It would appear then that the *Trichodesmium* P-Transport model is a tenable and testable model to describe the formation and maintenance of *Trichodesmium* blooms in the oligotrophic Pacific Ocean. Ecological predictions would include: (1) pre-bloom *Trichodesmium* cells located near the base of the euphotic zone but migrating upward (i.e., positively buoyant), should have low C:P ratios, high cellular phosphate conent, low carbohydrate and a high density of gas vacuoles; (2) post-bloom *Trichodesmium* cells located near the sea surface but migrating downward (i.e., negatively buoyant) should have high C:P ratios, no cellular polyphosphate, high carbohydrate and no gas vacuoles. The respective positively and negatively buoyant colonies could be collected using either paired "upward-looking" and "downward-looking" sediment traps (Smith et al. (1989)) or by shipboard discrimination of buoyancy characteristics of net-collected samples (Villareal and Carpenter (1990)). Furthermore, if the source of P required to support the bloom is actually derived from subeuphotic zone sources, as hypothesized in both of our ecosystem models, then the abundance of the twin cosmogenic radionuclides, ^{32}P and ^{33}P (Lal and Lee (1988), Lal et al. (1988)), of the total cellular P of a surface ocean *Trichodesmium* bloom should be closer to values of the total dissolved P pool at 125-150 m than to values predicted from trophospheric injection. These measurements would be a crucial test of the N-P uncoupling hypothesis which we hypothesize is an absolute prerequisite for *Trichodesmium* bloom formation.

5. Acknowledgements

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BUOYANCY REGULATION AND VERTICAL MIGRATION OF *TRICHODESMIUM*: A COMPUTER-MODEL PREDICTION

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ABSTRACT. In this paper the buoyancy regulation and vertical migration of three different *Trichodesmium* spp. was simulated by means of a computer model. The simulations showed that the colonies can sink to depths of 100 to 200 m, although, in general, the buoyancy regulation will keep the colonies in the upper water layers. The simulation also showed that nitrogen fixing colonies are more prone to photoinhibition and, as a consequence. will form surface blooms, which get trapped at the surface. This may cause the death of part of the population.

1. Introduction

In the previous chapter by Walsby the mechanisms of buoyancy regulation were discussed. Although the mechanism are well described. it is difficult to predict how the buoyant state of a cyanobacterium will change in response to the continuously changing conditions of light or nutrients that an organism encounters as it sinks or floats through the water column. In order to understand this, Kromkamp and Walsby (1990) developed a computer model that simulates changes in buoyancy in successive diel cycles. In the following section we will briefly discuss this model and some of its results and then apply it to what is known about the buoyancy of different *Trichodesmium spp.*

1.1. THE MODEL

The model makes predictions on the movements of a filament or colony as its floats up or sinks down in a column of water, adjusting its buoyancy in response to irradiance. nutrient concentration, and other factors. The assumption is first made that during the course of the day time, the irradiance at the surface can be described by a sine function of the maximum irradiance at noon (Kirk, 1983). From the irradiance at the surface, the irradiance at the depth

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of the organism is then calculated by means of negative exponential expression described by the attenuation of light (Lambert-Beer equation). The organism is then assumed to change its buoyant density in response to this irradiance, as described by the hyperbolic function:

$$d\rho/dt = c_1[I/(I + K_I)] - c_3 \quad (1)$$

where $d\rho/dt$ is the change in density with time (kg m^{-3} h^{-3}), c_1 is the maximum rate of density change with time, K_I is the irradiance at which the rate of density increase is half the maximal rate, I is the average irradiance and c_3 the minimal rate of density decrease. The values of these coefficients have been determined in a series of experiments on *Oscillatoria agardhii* (Kromkamp & Walsby, 1990). In the same investigation it was found that when samples that had been incubated at different irradiances were transferred to the dark, they decreased in density at a linear rate that was dependent upon the previous light history (I_a), given by the expression

$$d\rho/dt = c_2 I_a - c_3 \quad (2)$$

where c_2 is a light-dependent rate coefficient describing the change in density with time. Equations (1) and (2) were combined to give the overall rate of density change of an organism, at depth z, experiencing the irradiance I_z:

$$\rho_2 = \rho_1 + c_1[(I_z/(I_z + K_I)] - c_2 I_a - c_3 \quad (3)$$

where I_z is the irradiance at depth z. The new density is then used in the Stokes's equation to compute the new sinking velocity (v):

$$v = 2gr^2(\rho_c - \rho)A/(9\phi n) \quad (4)$$

where r is the radius of the colony ρ_c and ρ the density of the colony and water respectively, A the ratio of cell volume to colony volume, ϕ the form resistance and n the viscosity. The model also takes into account changes in viscosity caused by different temperatures, but the changes caused by this are only small and will not be considered here. As stated above, the rate of density change in the dark is also dependent upon the previous light history (see Kromkamp and Walsby, 1990, for details). Iterative calculations lead to a series of predictions for the depth occupied by the organism, its physiological state density and sinking or floating velocity. The calculations can be updated at any required time interval (we used 5 minutes) and the pattern of change can then be followed throughout the day and night.

The model predicts vertical migration as a consequence of changes in density which are solely caused by changes in ballast. The possible effects of turgor induced gas vesicle collapse or regulation of gas vesicle content are not specifically incorporated in the model. There are numerous cyanobacteria which have gas vesicles which are too strong to be collapsed by turgor pressure and regulation of gas vesicle content is probably a slow process, taking days (Kromkamp et al., 1986; Konopka et al., 1987)

Without a model one can make simple predictions about the movements of cyanobacteria. For example, a buoyant colony that has spent the night at the surface will, after exposure to light during the day time, lose buoyancy and sink. But how long will it take to respond? A

colony that has responded and lost its buoyancy will sink into depths where the irradiance is low, respond by gaining buoyancy, and float up again. But how long will it take to do this? Will it arrive back at the surface again that day, and if so, at what time? How do colony size and nutrient limitation affect the outcome. Our model permits quantitative answers to be given to these questions.

The predicted vertical migration of a colony of 300 µm diameter starting at dawn (t = 0) at a depth of 3m is shown in Fig. 1. Because the organism was buoyant, it floated to the surface where it stayed for 3h, before it lost its buoyancy and sank. Fig. 1 also shows that despite the 12h light period, the colony received only 6h of light during 24h.

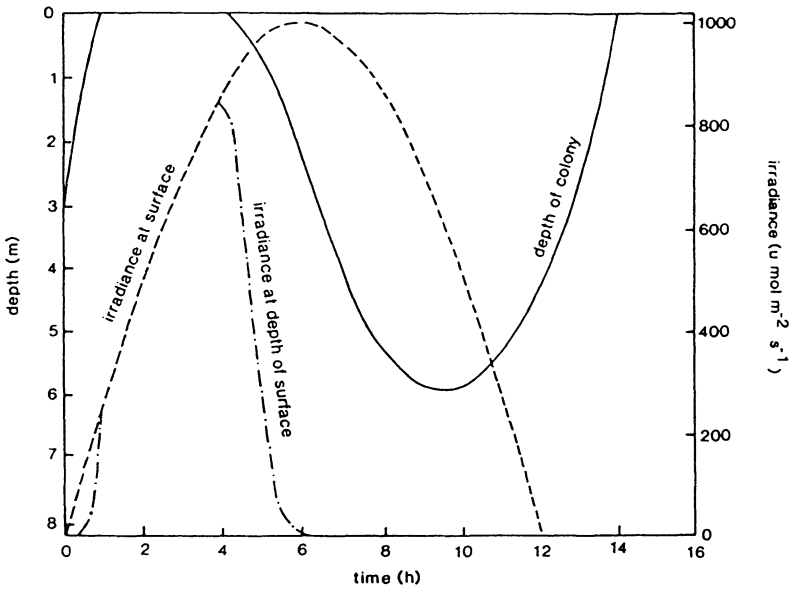

Fig. 1. Simulation of vertical migration of a colony with a diameter of 300µm. k_d (attenuation coefficient) = 2 m^{-1}. max. irradiance at noon is 1000 µE m^{-2} s^{-1}. starting density 980 $kg.m^{-3}$. minimum and maximum density 955 and 1070 kg m^{-3} respectively 12h light:12h dark period. (From Kromkamp and Walsby, 1990).

The model has also been used to the simulate the vertical migration of a population that had been randomly mixed and subsequently encountered calm conditions (Fig. 2). As can be seen, all the colonies floated to the lake surface and a large surface bloom formed. The colonies in the bloom started to sink 6h after sunrise (t = 0) and temporarily formed a layer at about 5m. This layer moved upwards and a surface bloom formed 2h after sunset. The resulting pattern of vertical migration is, of course, very much dependent upon the colony size. A significant finding of this model is that a surface bloom can be quite dynamic. Colonies leaving the surface are replaced by colonies floating up. Colonies coming from depth reach the surface later in the day and accumulate ballast more quickly than colonies at the surface at sunrise. This is due to higher irradiances later during the day. The result is that colonies converge at a particular depth giving the temporary appearance of a stratified layer.

This is caused solely by colonies moving up and down and should not be interpreted as a migration to a 'preferred depth'. In fact, colonies can hardly stay at a particular depth, due to their relatively large sinking and floating velocities (a consequence of Stokes' law), which will cause them to overshoot or undershoot a particular depth.

The model has also been used to investigate the effect of colony size and nutrient-limitation on migration. It has been shown that nutrient-limited colonies may sink to greater depths, which are often richer in nutrients. With respect to colony size it was found that if colonies were larger than a certain diameter, the depth reached was independent of colony size.

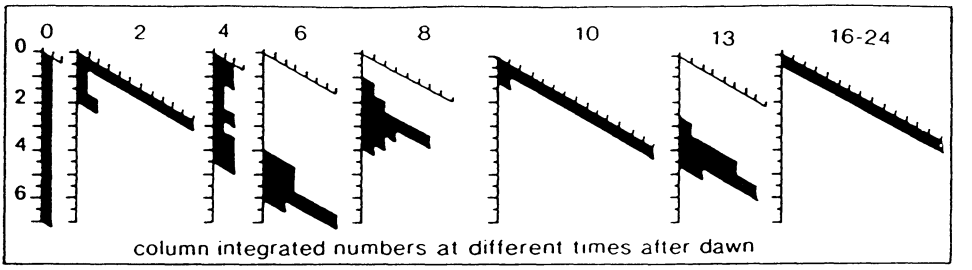

Fig. 2. Simulation of colony movement after turbulence ceased at sunrise at the start of the simulation (it was assumed there were no colonies below 10m). The histograms show the colony distribution at different times of the day. See Fig. 1 for details of run conditions. (From Kromkamp and Walsby, 1990).

2. Buoyancy regulation in *Trichodesmium*: a computer simulation

Buoyancy regulation is documented for *Trichodesmium* (Villareal and Carpenter, 1990) and it seems very likely that buoyancy density changes in these organisms are caused by ballast changes. Our model is based on actual measurements on density change by a planktonic cyanobacterium, *Oscillatoria agardhii*, in culture. The organism studied adjusts its buoyancy by synthesizing ballast (in high irradiance) or catabolizing carbohydrate (in low irradiance) (Utkilen et al., 1985). This mechanism appears to be used by all cyanobacteria (Kromkamp et al., 1986; Konopka et al., 1987. Some cyanobacteria with rather weak gas vesicles may additionally control their buoyancy by gas vesicle collapse under turgor (Oliver & Walsby, 1984; Kinsman et al., 1991). The outcome of this, buoyancy loss at high irradiance, is basically the same, although the kinetics may differ. *Trichodesmium* would not use the turgor collapse mechanism because its gas vesicles are too strong: it may be expected to respond like the *Oscillatoria* originally used to formulate the model.

We investigated the buoyancy regulation in three different *Trichodesmium* spp., *T. thiebautii* has rather spherical colonies, whereas *T. contortum* and *T. erythraeum* form fusiform colonies (Walsby, 1978). The rather flat colonies of *T. erythraeum* have rather smoother ends than the tufted, ragged ends of colonies of *T. contortum*. Hence, the different colonies will have different form resistances. The vertical migration of these colonies were computed, assuming a form resistance of 1 for *T. thiebautii*, 2 for *T. erythraeum* and 2.5 for

T. contortum. The rate constants c_1, c_2, c_3 and K_I were 0.132 kg m^{-3} min^{-1}, 7.67x10^{-5} kg m^{-3} min^{-1}(µE m^{-2} s^{-1})$^{-1}$, 0.023 kg m^{-3} min^{-1} and 25 µE m^{-2} s^{-1} (see the model description for explanation of these coefficients). Using the data on sinking velocities from Walsby (1978) and taking reasonable values for the form resistances of the different *Trichodesmium* colonies (from Reynolds et al., 1978) it was calculated that the cell volume is respectively 5, 10 and 3% of the colony volume. This is more than previously found for freshwater cyanobacteria (e.g. Walsby and McAllister, 1987), but filaments of *Trichodesmium* seem to be more tightly packed than their freshwater counterparts, a possible adaptation to nitrogen fixation. All three species showed the same change in excess density with time (a consequence of the same rate constants, not shown). The vertical migration of *T. thiebautii* and *T. erythraeum* was similar. Both colonies sank to approx. 35 m depth. The sinking velocities varied between 0 and 1 mm s^{-1}, whereas the floating velocities ranged between 0 and 0.4 mm s^{-1}. This agrees well with the observation made by Walsby (1978). Due to a higher form resistance and lower proportion of cells in the colony, colonies of *T. contortum* only migrated a couple of meters (Fig. 3).

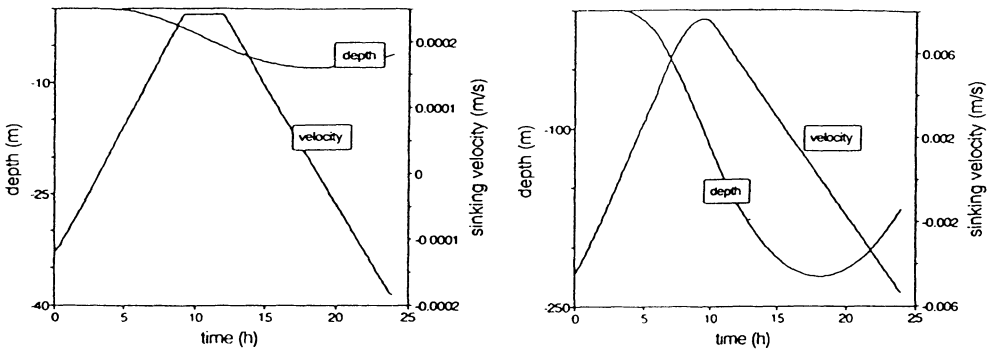

Fig. 3. Influence of form resistance on vertical migration of two different species of *Trichodesmium*. Minimum and maximum density respectively 970 and 1035 kg m^{-3}. K_d = 0.07 m^{-1}. Colony radius 500 µm. Starting density: 980 kg m^{-3}. Light-dark cycle: 12h:12h. Maximum irradiance at noon (t = 12 h) 1500 µE m^{-2}. Left: *T. contortum*. A = 0.03, r = 2.5. Right: *T. thiebautii*. A = 0.05, r = 1. For further details see text.

The effect of colony size on vertical migration was also investigated for a colony conform *T. thiebautii*, Fig. 4). For a colony with a diameter of 500 µm, the maximum sinking velocity was 1 mm s^{-1} (86 m day^{-1}). This agrees well with the velocities observed by Walsby (1978). The maximum density was reached about 9 h after sunrise. The depth reached was approximately 35 m. With a radius of 1000 µm the colony sank to a depth of approx. 150 m. The maximum density was reached about 10 h after dawn and the sinking velocities fluctuated between -2 and 4 mm s^{-1}, i.e. just outside the range observed by Walsby (1978). When the colony radius was 1500 µm, the colonies reached sinking velocities up to 7.5 mm s^{-1}. With still larger colony sizes, the depth reached did not increase, but the colonies started to float up earlier (not shown). These oscillations around neutral density were caused

because the changes in ballast were not very large: the colonies moved into and out of the euphotic zone too quickly to cause the large changes in density observed with the smaller colonies.

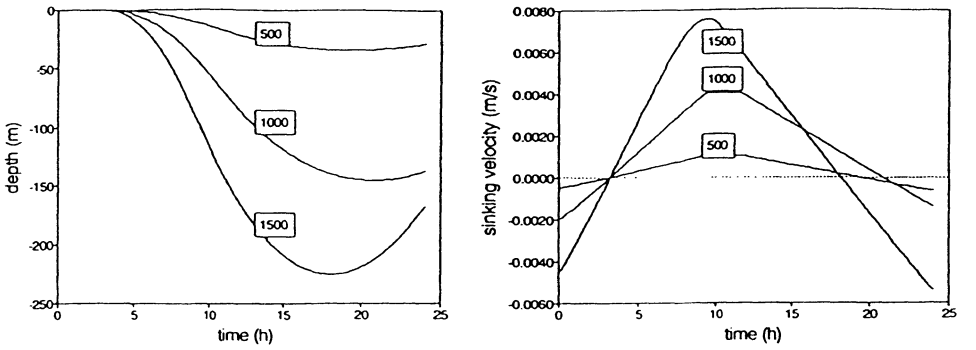

Fig. 4. Influence of colony size on vertical migration of *T. thiebautii*. For details of run conditions see Fig. 3. K_d=0.05 m^{-1}. The effective colony radii were 500, 1000 and 1500 µm. Left: vertical migration of the three different colonies. Right: the associated sinking and floating velocities.

3. The influence of nitrogen fixation on vertical migration

The predictions of the model are quite sensitive to the value of the rate constants, and these may be a function of the physiological state of the organisms. The rate constants used were determined with light-limited continuous cultures of the cyanobacterium *Oscillatoria agardhii* PCC7821, isolated from lake Gjersjøen in Norway. Hence, it is not very useful to run the model with the rate constants described in rather transparent water found in the oceans. It is assumed that the attenuation coefficient of the water was 0.25 m^{-1} (1% depth at approximately 15 m).

Nitrogen fixation makes photosynthesis less efficient, because the energy generated by the light reactions are used partly to reduce molecular nitrogen. This means that the maximum rate of photosynthesis is not changed but that of the amount of photosynthetic energy produced and stored as (carbohydrate) ballast is reduced, i.e. the value of K_I is larger. We changed its value from 25 to 35 µE m^{-2} s^{-1} when simulating a nitrogen fixing population. Also, it was assumed that the rate of respiration increased during N_2-fixing conditions. Hence, the rate constant c_2 was increased from 7.67×10^{-5} to 10×10^{-5} kg m^{-3} min^{-1}(µE m^{-2} s^{-1})$^{-1}$. The results of this will be that the rate of density accumulation will be less and the rate of density decrease in the dark will increase.

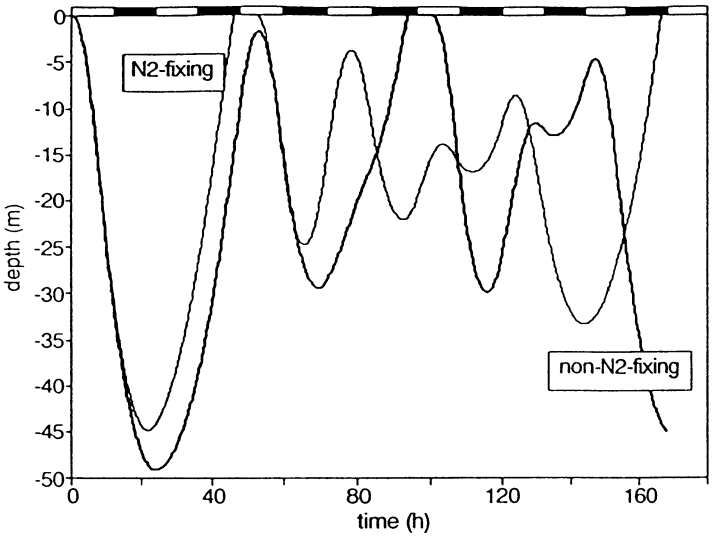

Fig. 5. Influence of nitrogen source on vertical migration of *T. thiebautii*. Details see Fig. 3 and text. K_d = 0.25 m^{-1}. Bar at top denotes dark period.

This can be seen very clearly during the first day of the simulation (Fig. 5): the non-nitrogen fixing colony sinks to a depth of 49 m and is after 24 h at the next sunrise, nearly neutrally buoyant (not shown). The nitrogen fixing colony on the other hand sinks to only 45 m depth and becomes buoyant again after 21 h, floats up and reaches the surface just before sunrise at day 3. From day 3 onwards, a rather stable situations seems to have developed in which both the N_2-fixers and the non-fixers migrate up and down in the upper water layers. However, after about 140 h the N-fixers rise to surface. The results of the simulation are of course dependent upon the starting conditions. The main point of this simulation is that under both conditions the colonies tend to become concentrated in the upper water layers.

4. The influence of photoinhibition on vertical migration in *Trichodesmium*

As was stated above, the physiological state of the cell will be reflected in the rates of buoyant density change. Not investigated previously is the effect of photoinhibition on buoyancy regulation. If we run the model with the rate constants as determined with light-limited cultures (see above), but for an environment with high irradiances (i.e. a large euphotic zone), then the organisms will harvest too much light. Initially this can cause higher accumulation rates of ballast so that the maximum density is reached more quickly. This is, of course, dependent upon the starting depth of the colony. However, because the watercolumn is very transparent and the irradiance "seen" during the day will be high, this will increase the rate of density decrease, not because c_2 will change but because the previous light history will have a high numerical value. The result of this is that in the subsequent

days the rate of ballast increase will be lower than the initial value and the rate of density decrease in the dark will be larger. This behavior can be expected when photoinhibition occurs: i.e. photorespiration will cause a decrease in the density and an increased rate of ballast catabolism. We have simulated this situation by following a colony for seven days at non-N_2-fixing conditions and during N_2-fixing conditions. For the latter case K_I was changed from 25 to 35 μE m^{-2} s^{-1} and c_2 was changed from 7.67×10^{-5} to 10^{-4} kg m^{-3} h^{-1}(μE m^{-2} s^{-1})$^{-1}$. The results are shown in Fig. 6.

4.1. COLONIES NOT FIXING N_2

In the next simulation the colonies were neutrally buoyant to start with; hence they lost buoyancy immediately and sank to approximately 50 m depth before regaining positive buoyancy. The second day they oscillated around a depth of 45 m. During the third and fourth day the colonies migrated downwards to a depth of 115 m before floating up again to a depth of 50 m. Colonies near the surface, which were very buoyant to start with, showed a different pattern: because it took time to negate the excess gas vesicle volume by carbohydrate ballast synthesis the colonies would experience high irradiances during the day: because of this light history it becomes more difficult to loose buoyancy and these colonies tended to become trapped at the surface after 4 days (not shown).

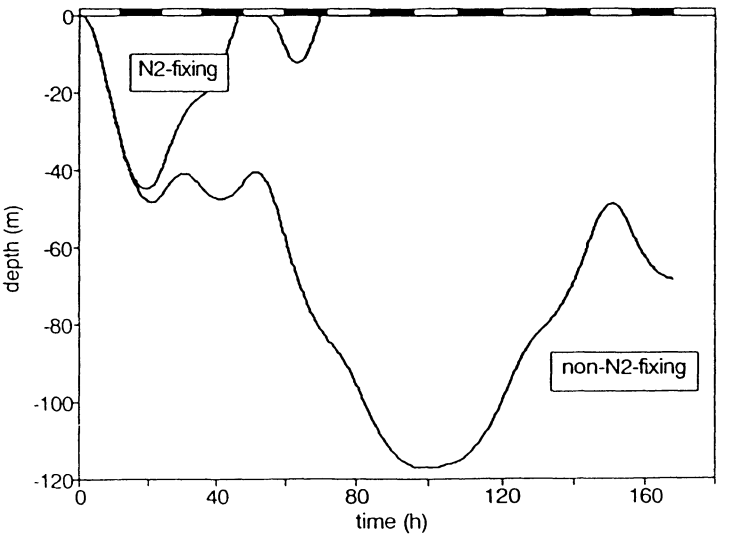

Fig. 6. Influence of photoinhibition on vertical migration in non-N_2 and N_2-fixing colonies of *T. thiebautii*. K_d = 0.05 m^{-1}. Bar at top denotes dark period.

4.2. NITROGEN FIXING COLONIES

As was argued earlier, N_2-fixation is likely to lead to a lower rate of ballast accumulation with increasing irradiance and to a higher rate of ballast catabolism due to a higher rate of respiration. N_2-fixing colonies sank to approximately the same depth as the non-fixing ones, but they floated upwards more quickly during the latter part of the first night. During day 2 they floated upwards and reached the surface just before sunrise at day 3. During this day, it took several hours to synthesize enough ballast to loose buoyancy and the colonies migrated only to a depth of approx. 10 m before floating to the surface again. Because of the light history, the colonies became trapped at the surface the next day (day 4) and were not able to loose buoyancy in the following three days. If this actually happened in the field, it is likely that a substantial part of the population would die. Nitrogen fixing colonies seem more prone to photoinhibition and formation of surface scums than colonies growing on nitrate or ammonia, and under conditions of photoinhibition would probably loose a greater part of the population due to the deleterious effects of prolonged photoinhibition.

5. Conclusions

The predictions of our model may be useful in understanding the pattern of vertical migration and distribution in *Trichodesmium* populations. Naturally, more accurate predictions could be made by first determining in experiments the rate constants. More measurement are needed of the sort made by Villareal & Carpenter (1989), accompanied by determinations of buoyant density by centrifugation on percoll gradients of colonies or filaments exposed to different irradiances.

Despite the uncertainties mentioned some useful conclusions can be drawn about the disadvantages and advantages of buoyancy regulation.

Disadvantages:

1. The formation of colonies creates inherent disadvantages like selfshading within the colony, a reduced availability of nutrients etc.
2. Due to the high floating velocities associated with colonies, the formation of surface blooms of buoyant colonies can occur. As shown above, photoinhibition can lead to death of part of the population. Nitrogen fixing colonies seem especially prone to photoinhibition.
3. In order to be able to float, cells have to synthesize gas vesicles. So a large proportion of the energy and nutrients will be converted to a (proteinaceous) structure which itself does not have any catalytic activity (the gas vesicle protein can be 5-15% of the total cell volume, Kromkamp et al., 1988, Oliver et al., 1985). This is especially true for *Trichodesmium* due to the narrowness of its gas vesicles.

Advantages associated with buoyancy regulation:

1. The colonies can stay in or near the euphotic zone.
2. When mixed down, they can float up rapidly.
3. As shown in Fig. 4, the colonies can by regulation buoyancy reach great depths, which may be more nutrient rich. This may have implications for calculations of new production
4. The colony form may help in reducing grazing pressure.

In spite of the disadvantages associated with buoyancy regulation and colony formation the trade off must be positive, considering the ubiquitous abundance of bloomforming cyanobacteria.

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MOLECULAR BIOLOGY OF NITROGEN FIXATION IN NATURAL POPULATIONS OF MARINE CYANOBACTERIA

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ABSTRACT. Our understanding of the factors that regulate nitrogen fixation in cyanobacteria is limited by our knowledge of aspects of the molecular biology of natural populations. *Trichodesmium*, a non-heterocystous cyanobacterium which is the dominant free-living diazotroph in open ocean waters, is unusual in that it fixes nitrogen aerobically without heterocysts. Furthermore, it has been shown that *Trichodesmium* only fixes nitrogen during the day, when photosynthetically active. Studies have been undertaken to determine the molecular and biochemical characteristics that allow nitrogen fixation and photosynthesis to co-occur, and to understand the factors regulating the activity of nitrogenase. This paper presents an overview of the results of these studies.

1. Introduction

The growth and activity of all living things are determined by the characteristics of genes and the way they are expressed in functioning enzymes. Our understanding of the way organisms function is dependent upon sufficient knowledge of these molecular characteristics and processes, and conversely the use of molecular probes can be an important tool for assessing the physiological state and the factors regulating biological activities of organisms in nature.

Cyanobacteria are important microbial components of terrestrial and aquatic ecosystems, and since they perform oxygenic photosynthesis they are also good models for the study of the regulation of photosynthesis. As a result, we know much about the molecular biology, biochemistry and physiology of the cyanobacteria, and there are numerous excellent reviews (Doolittle 1979, Carr and Whitton 1982, Rogers and Gallon 1988, Fay and Van Baalen 1987). In contrast, we know relatively little about the regulation of growth and activity of cyanobacteria in nature. Doolittle (1979) suggested that it should be possible to integrate ecological studies of population dynamics with molecular data on the role of environmental factors in regulating gene expression to develop essentially a new discipline which he termed "molecular ecology". Despite this far-sighted prediction, this new area of research is still, twelve years later, only in its infancy. The application of molecular biology to problems in marine science has been reviewed recently (Falkowski and Laroche 1991). Most of the microbiological studies in marine sciences have addressed taxonomic questions (e.g. DeLong et al. 1989, Giovannoni et al. 1990), or physiological questions using freshwater or marine cultures. Studies of the regulation of activities of natural populations of cyanobacteria at the molecular level are still few.

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The ability to fix atmospheric nitrogen is ecologically important and biologically one of the most interesting capabilities of the cyanobacteria. Although not all cyanobacterial species have the ability to fix nitrogen, the capability of nitrogen fixation is distributed in representatives of all taxonomic groups of the cyanobacteria (Rippka et al. 1979, Sprent and Sprent 1990). The ecology, biochemistry and molecular biology of nitrogen fixation in the cyanobacteria have been well-studied (reviewed by Paerl 1990, Gallon and Chaplin 1988a,b, Haselkorn 1986a, Bothe 1982, Fogg 1982, Carpenter 1983, Paerl et al. 1981). However, few studies have attempted to use our understanding of the biochemistry/molecular biology of nitrogen fixation to further our knowledge of the factors regulating cyanobacterial nitrogen fixation in the environment. In this paper, I will review what is known about the molecular biology of nitrogen fixation in the marine cyanobacterium *Trichodesmium*. From an ecological perspective, *Trichodesmium* is important since it is believed to be the major nitrogen fixing cyanobacterium in the open ocean (Carpenter 1983). With regard to nitrogen fixation, it is the only species which evolves oxygen while fixing nitrogen (i.e during the light phase of the light-dark cycle), usually fixes nitrogen only during the light phase (Saino and Hattori 1978, Capone et al. 1990, Ohki and Fujita 1988), and does not form heterocysts.

2. Nitrogen Fixation

Nitrogen fixation is the reduction of atmospheric dinitrogen gas to ammonium. This reduction is catalyzed by the enzyme nitrogenase, which is found in diverse groups of prokaryotic microorganisms. This subject has been reviewed extensively (e.g. Postgate 1982, Sprent and Sprent 1990) and has been the subject of numerous symposia (e.g. Bothe et al. 1988). There is a comprehensive list of the literature reviews of nitrogen fixation presented by Gallon and Chaplin (1988a). It is not the aim of this paper to attempt to provide yet another review of the fundamentals of nitrogen fixation, but to discuss those aspects of the molecular biology of nitrogen fixation of particular importance to understanding nitrogen fixation in marine cyanobacteria.

Several characteristics of nitrogen fixation are of primary importance in determining the extent of nitrogen fixation in the sea: 1) the large amount of energy and reductant required for the reduction of dinitrogen to ammonium, 2) the sensitivity of nitrogenase to inactivation by oxygen, 3) the regulation of nitrogenase synthesis and activity by the presence of fixed nitrogen (NO_3^- and NH_4^+) and 4) the requirement for micronutrients such as molybdenum, vanadium and iron. These characteristics set physiological limits on the temporal and spatial distribution of nitrogenase activity in the sea.

It is generally accepted that the reduction of dinitrogen requires 15 molecules of ATP and 6-8 electrons per molecule of N_2 fixed (Postgate 1982). This relatively large energy demand dictates that nitrogenase activity must be coupled with an energy producing reaction such as photosynthesis or the metabolism of organic carbon compounds (Sprent and Sprent 1990). In spite of the apparent advantage of linking nitrogen fixation directly to the photosynthetic production of ATP and NADPH, the rapid inactivation of nitrogenase by oxygen makes the direct coupling of nitrogen fixation to oxygenic photosynthesis physiologically difficult (Gallon 1981). Some filamentous species circumvent this problem by producing specialized cells, the heterocysts, where the activity of the oxygen evolving photosystem II is limited, and an anaerobic environment is maintained (Haselkorn 1978). Although ATP demands can be met through cyclic photophosphorylation, the requirement for reductant must be met by transport of

fixed carbon or reductant from photosynthesizing, vegetative cells (Gallon and Chaplin 1988a). Interestingly, although the heterocystous cyanobacteria seem to have evolved a specialized system for solving the oxygen problem, some nitrogen fixing cyanobacterial species in the marine environment (primarily *Trichodesmium*) do not form heterocysts (Bryceson and Fay 1982, Stal and Krumbein 1981), and others are not filamentous (Duerr 1981, Waterbury et al. 1988, Spiller and Shanmugam 1987). Nitrogen fixation by nonheterocystous species is discussed elsewhere in this volume (Gallon and Stal), but briefly, it has been found that energy and reductant appear to be provided by carbon metabolism, not directly from photosynthesis, even in these species (Gallon and Chaplin 1988a, Maryan et al. 1986). Some of these species are capable of fixing nitrogen and evolving oxygen at the same time in the same cell when grown under continuous light, but most species limit nitrogen fixation to the dark phase when grown under an alternating light-dark regime (Khamees et al. 1987). The physiological mechanisms that make nitrogen fixation in the light possible still have not been characterized.

Our understanding of the molecular biology of the nitrogen fixation apparatus, the regulating factors, and biochemistry of nitrogen fixation is founded on the many excellent studies of nitrogen fixation in pure cultures of microorganisms such as *Klebsiella*, *Azotobacter*, *Gloeothece*, and *Anabaena* sp. PCC 7120. In the environment, microorganisms are seldom provided with the stability of environmental conditions which are provided in culture. As a result, our understanding of how and when microorganisms function, and what regulates their activity is inadequate for predictions of how microorganisms will respond to their environment or changes in their environment. Furthermore, natural populations are assemblages of many microorganisms, and the identity and diversity of microorganisms with the capability of nitrogen fixation cannot always be ascertained. To make matters worse, marine microorganisms often cannot be maintained in culture, making it impossible to perform experiments in the laboratory. And finally, in order to determine how nitrogen fixing activity is regulated in the environment, molecular tools including DNA probes and antibodies need to be obtained for organisms which often cannot be cultured. Using molecular biology techniques it is possible to determine 1) which types of organisms in microbial assemblages can fix nitrogen, 2) the environmental cues which lead to nitrogenase expression and activity, and 3) unique characteristics of the structure of the nitrogenase genes and proteins that may explain the capabilities and limitations of nitrogen fixing cyanobacteria in the environment. In the following sections I will present the results of several studies that address these issues in natural populations of marine nitrogen fixing microorganims and the culture of *Trichodesmium* sp. NIBB 1067.

3. Identification of Nitrogen Fixing Organisms

Nitrogenase is composed of two proteins: the Fe protein and the Mo-Fe protein. Both of these proteins are highly conserved, with the Fe protein being the most highly conserved. The Fe protein (57-72 kD) is composed of two identical subunits (usually 28-35 kD) encoded by the *nifH* gene, and the Mo-Fe protein (200-240 kD) is composed of two pairs of different subunits (50 and 60 kD) encoded by the *nifD* and *nifK* genes. The highly conserved nature of these proteins makes it possible to easily clone and sequence fragments of these genes for species comparisons (Zehr and McReynolds 1989, Zehr et al. 1990, Kirshtein et al. 1991). Zehr and McReynolds (1989), in a study of the nitrogenase genes of natural populations of *Trichodesmium*, synthesized degenerate (redundant) oligonucleotides which were used in conjunction with the polymerase chain reaction to amplify, clone and sequence a fragment of *nifH*. These degenerate primers (126 and 98-fold) were designed in regions where there were at

least 5 amino acid residues which were found in all *nif* sequences. Since every possible oligonucleotide which could encode for these conserved amino acid sequences is represented, the primers can be used to amplify any *nifH* gene, regardless of biased codon usage by different microorganisms. These primers were used to obtain sequence information from a variety of diazotrophs, including several species of cyanobacteria (Zehr et al. 1990, Kirshtein et al. 1991). The results showed that the deduced amino acid sequence and the DNA sequence reflect taxonomic grouping and can be used to differentiate between heterotrophs and cyanobacteria (Table 1, Kirshtein et al. 1991). This provides a useful tool for analyzing mixed assemblages of microorganisms from nature. For example, starting with DNA extracted from a microbial assemblage, amplification, cloning and sequencing can provide information on the type(s) of microorganisms that have the potential for nitrogen fixation, and the diversity of microorganisms present. This type of study is currently in progress to determine the number of strains of *Trichodesmium* found in natural assemblages. Thus far, no heterotrophic *nif* sequences have been found in *Trichodesmium* bundles, even though a diazotrophic bacterium has been cultured from a *Trichodesmium* assemblage (Kirshtein et al. 1991, Currin et al. 1990, Paerl et al. 1989). Apparently, this bacterium is not very abundant *in situ*. Using this technique it should also be possible to settle issues such as the potential of other marine cyanobacteria, such as the picoplankton, to fix nitrogen.

	Cyanobacteria				Heterotrophic Bacteria				Purple Bacterium	
	1	2	3	4	5	6	7	8	9	10
1. Trichodesmium	-	74	80	75	69	68	71	60	66	63
2. A. flos-aquae	74	-	78	75	66	66	67	69	64	61
3. Anabaena 7120	80	78	-	87	71	67	69	66	69	68
4. A. oscillariodes	75	75	87	-	70	68	66	64	67	64
5. A. vinelandii	69	66	71	70	-	77	75	77	78	74
6. Klebsiella sp.	68	66	67	68	77	-	78	75	65	63
7. M1	71	67	69	66	75	78	-	75	65	63
8. K. pneumoniae	60	69	66	64	77	77	75	-	78	75
9. R. phaseoli	66	64	69	67	78	68	65	78	-	77
10. R. capsulata	63	61	68	64	74	65	63	75	77	-

Table 1. Similarity of upstream 175 bp of DNA sequence from amplified fragment of *nifH* from a number of microorganisms. Numbers along top refer to species identified along left margin. Data obtained from GenBank, J. Zehr unpublished data, Kirhstein et al. 1991).

Another approach for identification of nitrogen fixing microorganisms is the use of antisera to the Fe protein of nitrogenase labelled with a fluorescent compound (Currin et al. 1990). In this approach, bacterial and cyanobacterial cells are identified microscopically using a fluorescent microscope, after permeabilizing the cell membrane and probing with nitrogenase antisera. The advantages of this technique are direct enumeration of cells containing nitrogenase, the disadvantages being that the protein must be expressed at the time of collection and preservation,

and the cell walls must be permeabilized. The enumeration of nitrogen fixing microorganisms, in conjunction with sequence information obtained by PCR, can provide detailed information on the diversity of microorganisms in microbial communities that are capable of N_2-fixation. Both of these approaches will be important in determining the diversity of N_2-fixing cyanobacteria in marine systems such as benthic mats, coral reefs, and the plankton.

4. Environmental Cues and Regulating Factors

Most of our knowledge of nitrogen fixation in the marine environment is based on measurements of the rate of nitrogen fixation directly using ^{15}N, or indirectly using the acetylene reduction technique. These measurements have provided us with a broad knowledge of the spatial and temporal distribution of nitrogenase activity (Capone and Carpenter 1982, Carpenter and Capone 1983). In some cases, experimental manipulations in conjunction with nitrogen fixation measurements can point to factors which can enhance or inhibit nitrogen fixation (e.g. Capone and Budin 1982, Capone 1982). When nitrogen fixation is not detected it is not clear whether 1) the genetic potential for nitrogen fixation is not present, 2) short term variability in environmental conditions or diel cycles reduced activity of the protein, or 3) conditions have led to repression of gene expression. The use of molecular probes to detect the presence of *nif* genes and their transcription, and antibodies to detect expression (and modification, see below), can provide an understanding of the mechanisms regulating nitrogen fixation activity, the environmental cues which lead to that regulation, and the length of time for response of the nitrogen fixation apparatus following a return of favorable conditions.

Briefly, nitrogen fixation can be regulated at several levels. First, in most heterocystous species there is a gene rearrangement that occurs within the structural *nif* operon that coincides with the development of the heterocyst, the major site of nitrogen fixation in these species (Golden et al. 1985). Although this characteristic is not strictly involved in regulation, it does coincide with the development of the nitrogen fixing cells. Second, transcription is probably regulated by environmental factors, such as oxygen, by as yet uncharacterized mechanisms similar to those found in *Klebsiella*. Third, the Fe protein of nitrogenase can be inactivated by an apparent modification (chemical or conformational change) that affects its electrophoretic mobility in SDS-PAGE gels (Kanemoto and Ludden 1984). Finally, the activity of nitrogenase can be directly regulated by biochemical conditions such as the supply of ATP.

In natural populations of *Trichodesmium*, nitrogenase activity is correlated with the light-dark cycle (Capone et al. 1990), as in many other natural populations of cyanobacteria (Horne 1979, Vanderhoef et al. 1975, Khamees et al. 1987, Griffiths et al. 1987, Storch et al. 1990). In contrast to other nonheterocystous cyanobacteria (Khamees et al. 1987, Griffiths et al. 1987), *Trichodesmium* fixes nitrogen primarily during the day (Capone et al. 1990). The reasons for limitation of nitrogen fixation activity to daylight hours are not clear, but this phenomenon, which poses a biological problem, is ecologically extremely important since it constrains the daily nitrogen fixation rate. The reasons for this diel regulation of nitrogenase activity could be related to photosynthetic oxygen production, diel changes in carbon and nitrogen metabolism, or physiological factors.

5. Regulation

5.1 REGULATION OF EXPRESSION

The regulation of *nif* transcription has been well studied in *Klebsiella*, *Rhizobium* and *Azotobacter* (see Postgate 1982). Due to the lack of a suitable genetic system in the nitrogen fixing cyanobacteria, less is known about the transcriptional regulation of *nif* in cyanobacteria (Haselkorn 1986b). The *nif* operon in *Klebsiella* is transcriptionally controlled through a cascade system involving the *ntr* and *nifLA* gene products (see Dixon 1984, Gallon and Chaplin 1988a). The *nifA* gene product is required for activation of the structural gene operon, but interestingly, the *nifL* gene product binds to the *nifA* product preventing activation of the *nifHDK* operon in the presence of oxygen (Dixon 1984). It is not known if there is a corresponding system in cyanobacteria. However, even if an analogous system existed, it is not clear how it would function in the presence of photosynthetic oxygen evolution. An alternate mechanism for the regulation of transcription by oxygen involves the supercoiling of DNA. The degree of supercoiling or negative supercoiling is regulated by the activities of DNA topoisomerase I and DNA topoisomerase II (DNA gyrase), respectively. The activities of these enzymes can be regulated by the presence of oxygen, and subsequently the efficiency of transcription altered (Kranz and Haselkorn 1986, Gallon and Chaplin 1988a). This mechanism has been suggested to be involved in the regulation of transcription of the *nif* operon in the unicellular cyanobacterium *Gloeothece* (Gallon and Chaplin 1988a). Whether this mechanism is important in regulating aerobic *nif* expression in *Gloethece*, as well as in filamentous nonheterocystous cyanobacteria such as *Trichodesmium*, remains to be determined.

Factors that result in favorable conditions for nitrogenase activity can be assessed in a fairly direct way by monitoring the levels of transcripts for the *nifHDK* genes themselves. Changes in abundance of transcripts can indicate when favorable conditions for nitrogenase expression occur, and help to determine the factors which affect the observed temporal and spatial variability in nitrogenase activity. Monitoring transcripts makes it possible to determine the first environmental cues that initiate the synthesis of nitrogenase. Huang et al. (1988) were able to demonstrate that the daily cycle of nitrogen fixation in *Synechococcus* RF-1 was regulated at the level of transcription. In the case of *Synechococcus* RF-1, fixation occurs only during the dark. In natural populations of *Trichodesmium*, which only fix nitrogen in the light, fixation in the morning is dependent on nitrogenase synthesis (Capone et al. 1990). It is probable that this synthesis of nitrogenase is regulated at least in part at the level of transcription, but this needs to be directly investigated by measuring transcript levels. In both cases, the reason for the daily cycle can be investigated by experimentally manipulating the system during the daily cycle and evaluating the effect on transcription. In this way, we can hopefully obtain the basis for daily cycles in nitrogenase synthesis: endogenous rhythms, effects of carbon or nitrogen metabolism, or oxygen repression.

The transcription of genes does not necessarily lead to nitrogen fixation activity, since changes in the external environment can inhibit nitrogen fixation at other levels as well. Since there exists a post-translational modification system for regulating the activity of the Fe protein, regulation of activity need not correspond to transcription.

5.2 REGULATION OF ACTIVITY

After nitrogenase proteins are synthesized, activity can still be regulated at other levels. In *Rhodospirillum* spp. and some *Azospirillum* spp., there is an enzyme which modifies the Fe protein by ADP-ribosylation (DRAT), which results in inactivation of the protein (Ludden and Burris 1978, Pope et al. 1985, Lowery et al. 1986, Fu et al. 1989) . The ribosylation affects the migration rate of the modified subunit in SDS-PAGE gels, so that the modification can be monitored by electrophoresis and Western blotting. The chemical modification has not been characterized in the cyanobacteria, although there is an apparent modification based on changes in mobility of the Fe protein in response to nitrogen, and oxygen (Reich and Boger 1989). Two protein bands cross-reacted with antisera raised against the product of the cloned fragment of *nifH* from *Trichodesmium* (Zehr et al. 1990, Ohki et al. 1991). The bands were about 2 kD different in apparent molecular mass, similar to the difference seen in other diazotrophs (Kanemoto and Ludden 1984, Stal and Bergman 1990, Reich and Boger 1989). However, in this paper, modification will be used to describe the change in mobility without implying any specific chemical change in the protein, since it has not been characterized in organisms other than *Rhodospirillum*. This change in mobility, although it responds similarly to the ribosylation in *Rhodospirillum*, may be a completely different phenomenon.

In *Trichodesmium*, nitrogenase activity ceases in the dark, and the protein is degraded slowly through the dark hours, virtually disappearing just prior to the return of daylight (Capone et al. 1990). These results were obtained with populations of *Trichodesmium* from the Caribbean Sea. The same results were obtained with populations from the Sargasso Sea (Fig. 1). The prior study compared the amount of protein on a per colony basis (Capone et al. 1990). In the Bermuda sample, it can be seen that there was not equal amounts of protein per colony (Fig. 1A). However, even when the gel was loaded with equal amounts of protein, the abundance of the Fe protein increased in the morning hours (Fig. 1C). The Fe protein was not apparent at 0500 hrs (Fig. 1B,C). The change in abundance of the Fe protein is apparent both on a colony and protein basis (Fig. 1). Clearly, the protein decreases in relative abundance during the diel cycle (Fig. 1). Immunolocalization data indicate a diel change in the distribution of nitrogenase within cells (Bergman and Carpenter 1991), but it is not possible to differentiate modified and unmodified protein with this technique. Preliminary data suggest that the natural populations do have the capacity to modify the Fe protein (data not shown), but it is not clear whether modification is involved during the day-night change in activity. Unfortunately, the resolution of the precast gradient gels used on shipboard did not resolve the modified/unmodified doublet well. Presumably, the Fe protein is modified in darkness, followed by slow degradation of the protein and renewed synthesis in the morning. The appearance of the protein in the morning, and the chloramphenicol sensitivity of nitrogenase activity in the morning indicate that protein synthesis determines the diel cycle of activity in natural populations (Capone et al. 1990).

In contrast to these results, the Fe protein in *Trichodesmium* sp. NIBB 1067 remains present throughout the day, but appears to be modified at night (Ohki et al., this volume). During the dark hours the protein is modified (slower electrophoretic mobility), and during the light, the protein is unmodified (faster electrophoretic mobility)(Ohki et al., this volume). The modification is correlated with activity of the protein: during the night there is no nitrogenase activity, and during the day, nitrogenase activity begins after an initial lag when the lights are turned on (Ohki et al., this volume). It will be interesting to determine the reasons for these apparent differences in the diel cycles between the culture and the natural populations. One

possible explanation is that the natural populations are growing much slower and fix sufficient nitrogen during the day for the nitrogen requirements for growth, which results in cessation of *nif* transcription. In the culture, inorganic nitrogen (2 mM nitrate, 20 uM ammonium) does not necessarily repress nitrogenase synthesis, as the modified protein is maintained in cultures under these conditions for over 15 generations (Ohki et al. 1991). However, availability of inorganic nitrogen does lead to modification and inactivation of the Fe protein (Ohki et al. 1991).

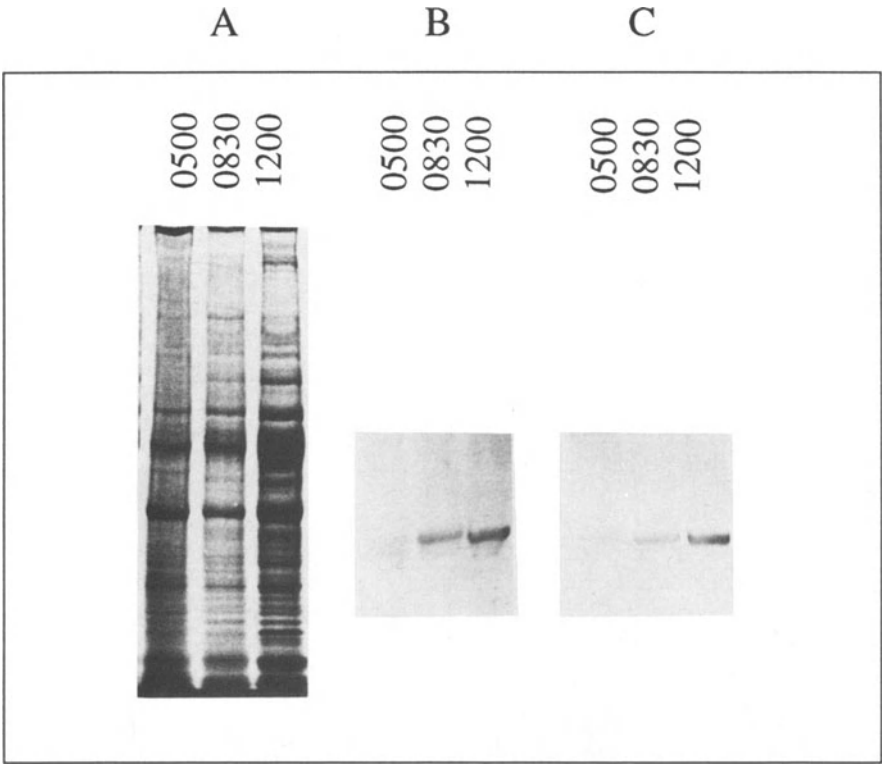

Fig. 1. Diel pattern of nitrogenase abundance in *Trichodesmium* spp. collected north of Bermuda, July 1990. Samples taken at 0500, 0830, 1200 hours. A. Coomassie blue stained protein gel; equal number of colonies loaded per lane. B. Western immunoblot probed with *Trichodesmium* nitrogenase antisera. Samples loaded with equal numbers of colonies. C. Western blot. Samples were loaded equal amounts of protein. Nitrogenase was identified with antisera raised against a fusion protein product of the *nifH* fragment amplified from natural populations of *Trichodesmium* (Zehr et al. 1990).

5.3 BIOCHEMICAL REGULATION

Transcriptional, translational and post-translational mechanisms are not the only factors regulating nitrogenase activity. Other biochemical factors such as the supply of ATP, may also regulate the activity of nitrogenase (Postgate 1982). In *Trichodesmium* NIBB 1067, nitrogenase activity ceases rapidly in darkness, before the Fe protein becomes modified (Ohki et al. 1991). The short term response to manipulations is often a cessation of nitrogenase activity which is not strictly correlated with presence of the modified Fe protein. This indicates that another mechanism is involved in the regulation of nitrogenase activity (Ohki et al. 1991).

6. Characteristics of *nif* genes

The structure of the *nif* genes themselves contain information on the regulation and function of nitrogenase. There are many regulatory genes and genes involved in maturation of the structural proteins in *Klebsiella* (Dixon 1984), but little is known about these genes in cyanobacteria. The characteristics of the structural *nif* genes themselves can provide information on the size of the nitrogenase proteins, as well as provide information on the similarity to the primary structure of other nitrogenase proteins. The *nif* genes, in particular *nifH*, can be present in multiple copies, even encoding a nitrogenase with different micronutrient requirements (V or Fe instead of Mo, reviewed by Joerger and Bishop 1988). This has particular significance in the marine environment since molybdenum availability has been hypothesized to limit nitrogen fixation in the sea (Howarth and Cole 1985). And finally, the structural *nif* genes undergo a gene rearrangement that is a characteristic of the differentiation of the specialized cells for nitrogen fixation, the heterocysts (Golden et al. 1985, Haselkorn 1986b).

In many nitrogen fixing microorganisms, the structural genes for nitrogenase, *nifH*, *D*, and *K*, are contiguously arranged. In particular, in the cyanobacteria, two arrangements have been described: 1) in most filamentous nonheterocystous cyanobacteria, unicellular cyanobacteria, and the heterocysts of heterocystous cyanobacteria the structural *nif* genes are contiguous, and 2) in the vegetative cells of most heterocystous cyanobacteria, the structural *nif* genes are interrupted by an 11 kb transposon near the downstream end of *nifD* (Golden et al. 1985). Although this transposon is removed during differentiation of the heterocyst, it is not believed to be directly involved in regulating transcription. However, the finding that the heterocystous cyanobacterium *Fischerella*, which has rather different characteristics of nitrogenase expression and heterocysts, has contiguous *nif* genes led to the conclusion that the gene rearrangement may reflect differences in regulation of expression of nitrogenase activity (Saville et al. 1987).

Unfortunately, the characterization of the *nif* genes usually requires relatively large amounts of cells for DNA extraction and purification. Zehr and McReynolds (1989) were able to sequence a fragment of the *nifH* gene of *Trichodesmium* starting with a few nanograms of DNA, but this technique is not easily used to obtain complete gene sequence data. Using DNA purified from the culture of *Trichodesmium* NIBB 1067, it was possible to map the structural *nif* operon (Zehr et al. 1991), using fragments of *nifH,D* and *K* from *Anabaena* sp. PCC 7120 (generously provided by R. Haselkorn). The results showed that the structure of the *nif* operon is similar in physical arrangement to that of *Anabaena* sp. 7120 (Fig. 2). Furthermore, there was not a gene rearrangement, such as that in *Anabaena sp,* in nitrogen fixing vs. non-nitrogen fixing cultures (Zehr et al. 1991).

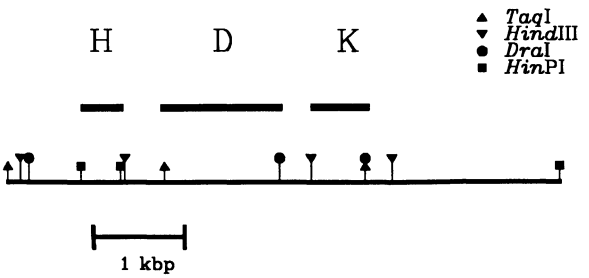

Fig. 2. Restriction map of the structural *nif* operon in *Trichodesmium* sp. NIBB 1067. Horizontal bars show smallest fragments which hybridize to *nifH*, *D* and *K* probes from *Anabaena* sp. 7120. The same map was obtained from cultures grown under both nitrogen fixing and non-nitrogen fixing conditions. (Zehr et al.,1991).

There were not multiple copies of any of the structural genes (Zehr et al. 1991). Since genes for the alternative nitrogenases may not have hybridized at the stringency used it is not yet clear whether alternative nitrogenases are present (Zehr et al. 1991). Although the restriction map constructed from the data showed that the genes also must be similar in size to that of *Anabaena* sp. 7120, the resolution of the map could allow for a variation large enough to account for the larger size of the protein on SDS-PAGE gels (43 kD, Ohki et al. 1991). Although it was not possible to map the *nif* genes from natural populations of *Trichodesmium* from the Caribbean, it was previously shown that the culture of *Trichodesmium* sp. NIBB 1067 and natural populations of *Trichodesmium* in the Caribbean were closely related, based on DNA similarity (98% on DNA and protein level) of the amplified *nif*H fragment (Fig. 3, Zehr et al. 1990). This similarity implies that the culture *Trichodesmium* sp. NIBB 1067 is closely related to the strains found in natural populations. Therefore, we can use this culture as a model system for genetic and physiological experiments that are difficult to perform with natural populations.

```
      E  R  G  A  V  E  D  V  E  L  D  E  V  L  K  P  G  F  G
TrN:  GAGCGCGGTGCAGTTGAAGACGTAGAACTAGATGAAGTATTGAAACCAGGTTTCG
TrC:    A T                          CC          G
          L                           Q

   G  I  K  C  V  E  S  G  G  P  E  P  G  V  G  C  A  G  R  G
GTGGCATTAAGTGTGTTGAGTCTGGTGGTCCTGAGCCTGGTGTAGGTTGCGCTGGCCGTG

   I  I  T  A  I  N  F  L
GTATTATTACTGCTATCAACTTCCTA
                      T
```

Fig. 3. Comparison of DNA and deduced amino acid sequences of amplified fragment of *nifH*. TrN= *Trichodesmium* sp. NIBB 1067. TrC=*Trichodesmium thiebautii*. The only differences were found in this region 75-216 bp from 5' end.

7. Aerobic Nitrogen Fixation

One of the interesting features of nitrogen fixation in *Trichodesmium* is the ability to fix N_2 aerobically without heterocysts. Therefore, one of the key biological questions is what are the characteristics that allow *Trichodesmium* to simultaneously evolve oxygen and fix nitrogen? One possibility is that the activities of nitrogen fixation and photosynthesis are restricted to separate cells, as in vegetative cells and heterocysts. This could occur in two different ways: 1) differential expression of nitrogenase and photosystem II components as in heterocystous species, or 2) restriction of nitrogenase activity to cells in an anaerobic microzone, such as sometimes occur in the center of colonies (Paerl and Bebout 1988). The hypothesis of Carpenter and Price (1976) was essentially a combination of these two: cells in the center of the colony were suggested to be differentiated. More recent results show that the photosynthetic pigment composition of central cells and peripheral cells is similar (Carpenter et al. 1990). A rearrangement of the *nif* structural genes as in the development of heterocysts would provide one indication of the development of cells with specialized function. However, the *nif* genes in *Trichodesmium* are contiguous and do not rearrange. Immunolocalization data does not support the hypothesis that cells in the interior of the colonies are responsible for the nitrogen fixation (Bergman and Carpenter 1991). Furthermore, highest nitrogen fixation rates per unit chlorophyll are obtained from the culture when in log growth phase and composed mostly of single trichomes (Ohki and Fujita 1988). Therefore, the capability of simultaneous oxygen evolution and nitrogen fixation must rely on biochemical or molecular mechanisms to avoid inactivation of nitrogenase by oxygen. Some of the biochemical features that could make this possible will be discussed elsewhere in this volume. The molecular characteristics described here, unfortunately, provide no obvious answers. The genes are similar in size and arrangement to those of other diazotrophs, with no apparent unusual characteristics. Studies have found no evidence for localization of nitrogenase, either within filaments, or within cells, although there may be interfilament variations (Bergman and Carpenter 1991). The only unique characteristic is the apparent size of the Fe protein on SDS-PAGE gels (Ohki et al. 1991, Bergman and Carpenter 1991). The resolution of the restriction map of the *nif* operon is not fine enough to detect the 150 nucleotide base pair difference required for the larger size (about 5 kD, 50 amino acids, 150 nucleotides). The Fe protein may yet be found to have unusual characteristics that may make the protein less sensitive to oxygen inactivation. The protein is modified in response to oxygen, being modified and inactivated within minutes (Fig. 4). Since the modification of the Fe protein has not been characterized, it could be that the modification is involved in protection, like the conformational protection of nitrogenase in *Azotobacter* (discussed by Postgate 1982). Incubation under nitrogen results in the protein becoming fully unmodified and presumably most active (Fig. 4). This apparent modification could be important in short-term responses to elevated oxygen levels, and deserves further study. However, both the modification in *Rhodospirillum* and conformational protection in *Azotobacter* result in temporary inactivation of the protein. Therefore, neither mechanism can explain simultaneous nitrogen fixation and oxygen evolution.

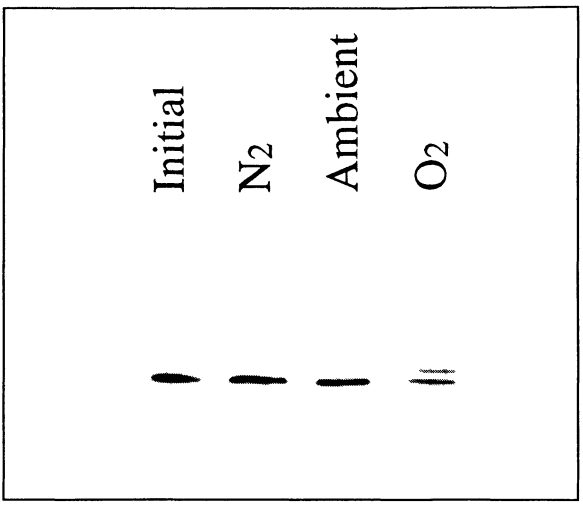

Fig. 4. Western blot showing the effect of oxygen on the Fe protein of nitrogenase from *Trichodesmium* sp. NIBB 1067. Samples were incubated under nitrogen, 20 % oxygen and 40 % oxygen in the light for 30 minutes. Nitrogenase was identified using antisera against the Fe protein of *Rhodospirillum rubrum*, generously provided by P. Ludden, University of Wisconsin (K. Ohki, J. Zehr, and Y. Fujita, unpub. data).

8. Summary

The bloom-forming non-heterocystous cyanobacterium *Trichodesmium* appears to be the most abundant free-living daizotroph in open ocean waters. It is peculiar in that 1) other nitrogen-fixing cyanobacteria are not also found in these waters where nitrogen concentrations are low, and 2) it has the ability to fix nitrogen aerobically without heterocysts. Furthermore, *Trichodesmium* will only fix nitrogen during the day while active in photosynthesis. The molecular biology of *Trichodesmium* has been investigated to understand how and why *Trichodesmium* fixes nitrogen during the day. Results have shown that 1) regulation of nitrogen fixation occurs at the level of protein synthesis, by modification of the Fe protein of nitrogenase, and by an uncharacterized mechanism that responds quickly to cessation of photosynthesis, 2) the structural *nif* genes have been found to be contiguous as in other non-heterocystous cyanobacteria, and do not undergo the rearrangements that have been characterized in most heterocystous cyanobacteria, and 3) multiple copies of the *nif* genes are not present, although it still may be that alternative *nif* operons exist. Proposed mechanisms for simultaneous nitrogen fixation and photosynthesis have relied upon localization of nitrogenase activity or differentiation of nitrogen-fixing and photosynthesizing cells. Results of work with natural populations and cultures of *Trichodesmium* indicate that this is not the case. The enigma can only be solved then, by an oxygen tolerance of *Trichodesmium* nitrogenase, or the presence of oxygen utilizing enzymes. Future research should be directed towards characterizing the primary structure of nitrogenase to identify unusual characteristics that may impart oxygen tolerance, as well as evaluating the potential for oxygen utilizing enzymes to protect nitrogenase from irreversible inactivation.

9. Acknowledgements

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PELAGIC *TRICHODESMIUM* (=*OSCILLATORIA*) IN THE GREAT BARRIER REEF REGION

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ABSTRACT. *Trichodesmium* (=*Oscillatoria*) blooms have long been recognized in waters of the Great Barrier Reef, Australia. Populations fluctuate episodically, forming conspicuous surface aggregations, some of considerable size. Proximate causes of these surface blooms are unknown. Further understanding of the role of *Trichodesmium* in the GBR system is constrained by lack of knowledge of non-aggregated distributions of *Trichodesmium*.

1. Introduction

Free-living cyanobacteria are the engine of primary production for the shelf sea ecosystem of the Great Barrier Reef (hereafter GBR). Although the physical structure of individual reefs is formed by corals and calcareous algae, the primary production which sustains the ecosystem largely comes from epilithic and epipsammic turfs of cyanobacteria (e.g. Barnes, 1983; Klumpp and McKinnon, 1989). On an areal basis, reefs cover 9 percent of the GBR shelf (Hopley et al., 1989), but contribute approximately 40 percent of shelf primary production (Furnas and Mitchell, 1989). Epilithic turf algal assemblages also make a significant contribution to reef nutrient dynamics through the fixation of gaseous di-nitrogen (Wilkinson et al., 1984; Larkum et al., 1988).

Phytoplankton in inter-reef waters are the major primary producers of the GBR system, contributing an estimated 60 percent of total shelf production (Furnas and Mitchell, 1989). Small phytoplankton in the picoplankton size class (<2 um) are, at most times, the predominant size group of pelagic primary producers (Furnas and Mitchell, 1989). The picoplantkon size fraction is numerically dominated by very small coccoidal cyanobacteria resembling *Synechococcus* (Furnas and Michell, 1986).

Pelagic cyanobacteria of the genus *Trichodesmium* (=*Oscillatoria*) comprise an episodically conspicuous element of the flora in the microplankton (>20 um) size fraction. Surface aggregations of *Trichodesmium* have been noted in GBR and northern Australasian waters from the beginning of European exploration of the GBR. Both Captain James Cook and one of his accompanying naturalists, Sir Joseph Banks noted blooms or slicks of *Trichodesmium* within the GBR lagoon and in the eastern Arafura Sea, just northwest of Torres Strait (Figure 1).

5 June, 1770 - From Banks' journal - near Townsville: *"Many clouds of a thin scum lay floating upon the water the same as we have before seen off Rio de Janiero; some few flying fish also."* (Beaglehole, 1962).

27 August, 1770 - From Banks' journal - eastern Arafura Sea: *"At dinner time we were alarmed afresh by the usual report of a shoal just ahead: it provd however to be no more than a bank or regular layer of a brownish color extending itself upon the sea, which indeed had very much the appearance of a shoal while at a distance. It was formed by innumerable small atoms each scarce 1/2*

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a line in length yet when lookd at in a microscope consisting of 30 or 40 tubes, each hollow and divided throughout the length into many cells by small partitions like the tubes of confervas; to which of the three kingdoms of nature they belong I am totally ignorant, I only guess that they are of a vegetable nature because on burning them I could perceive no animal smell." (Beaglehole,1962).

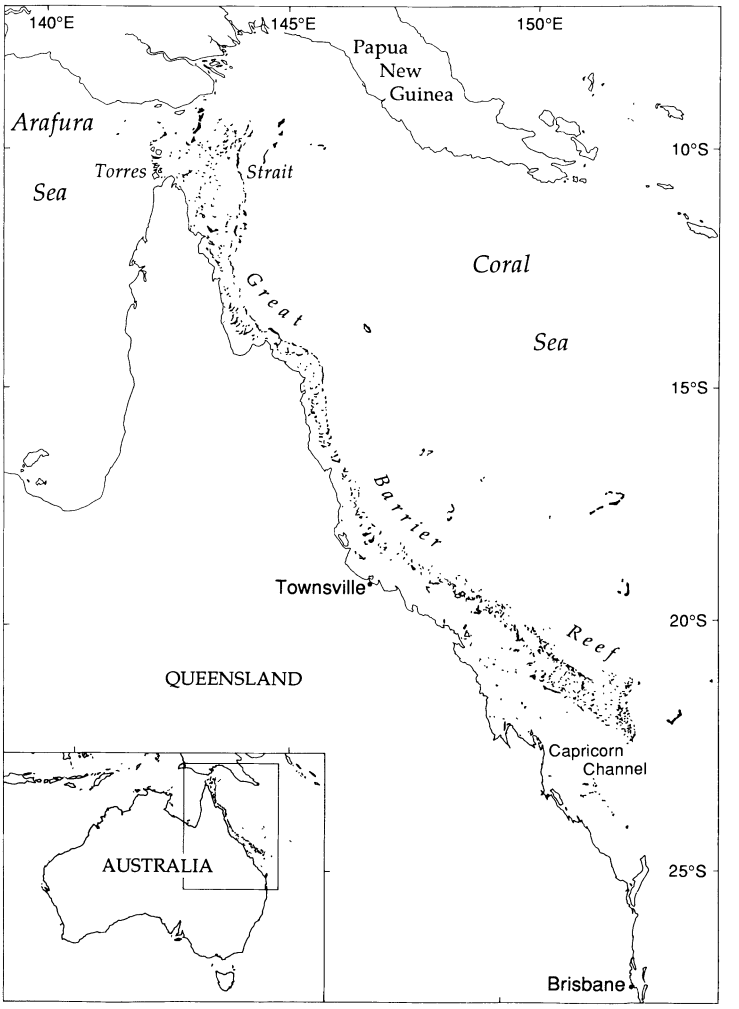

Figure 1. The Great Barrier Reef and eastern Arafura Sea

28 August 1770 - From Cook's journal - eastern Arafura Sea: *"The sea in many places is here cover'd with a kind of brown scum, such as sailors generally call spawn; upon or first seeing it, it alarm'd us thinking that we were among shoals, but we found the same depth of water where it was as in other places. Neither Mr. Banks nor Dr. Solander could tell what it was altho they had of it to examine."* (Beaglehole, 1955).

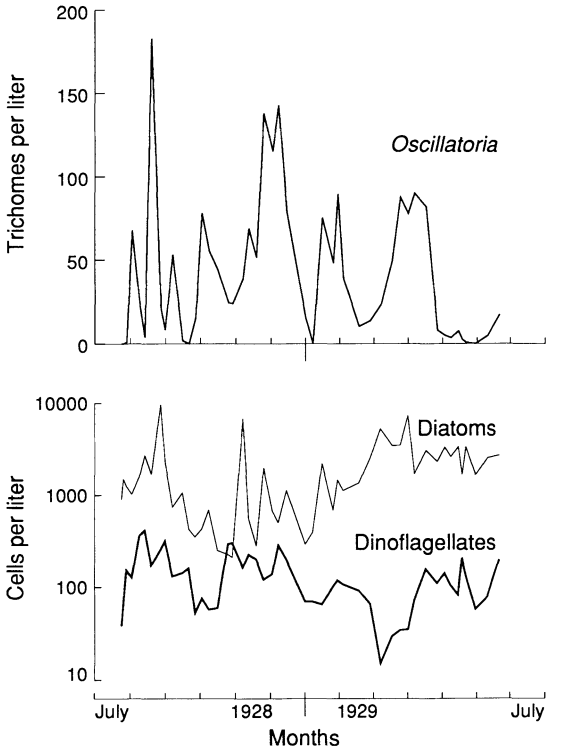

Figure 2. Fluctuations in depth-weighted mean water column concentrations of *Trichodesmium* (trichomes per liter), diatoms and dinoflagellates (cells per liter) near Low Islands over an annual cycle. Data were plotted from Marshall (1933).

Following these initial descriptions, numerous reports of floating *Trichodesmium* in the GBR region by scientists and lay observers have been published (some summarized by Cribb, 1969). Lucas (1919) made the first identification, naming material collected near Cooktown as *Trichodesmium scoboideum* Lucas. At the present time, only the two pan-tropical species of pelagic *Trichodesmium* (= *Oscillatoria*: *O. erythraeum* and *O. thiebauti*) are recognized (Marshall, 1933; Hallegraeff and Jeffrey, 1984).

As with many aspects of phytoplankton ecology in the GBR region, very little is known regarding the abundance of *Trichodesmium* populations, their temporal and spatial variability or the factors responsible for local changes in populations. Although *Trichodesmium* may be found at any time of the year within water samples collected at any depth throughout the GBR, attention has largely been drawn to the episodic surface aggregations within the GBR lagoon, a contiguous body of open water between the coast and the reef matrix. These aggregations or blooms occur throughout the year, but are most frequently observed between August and February.

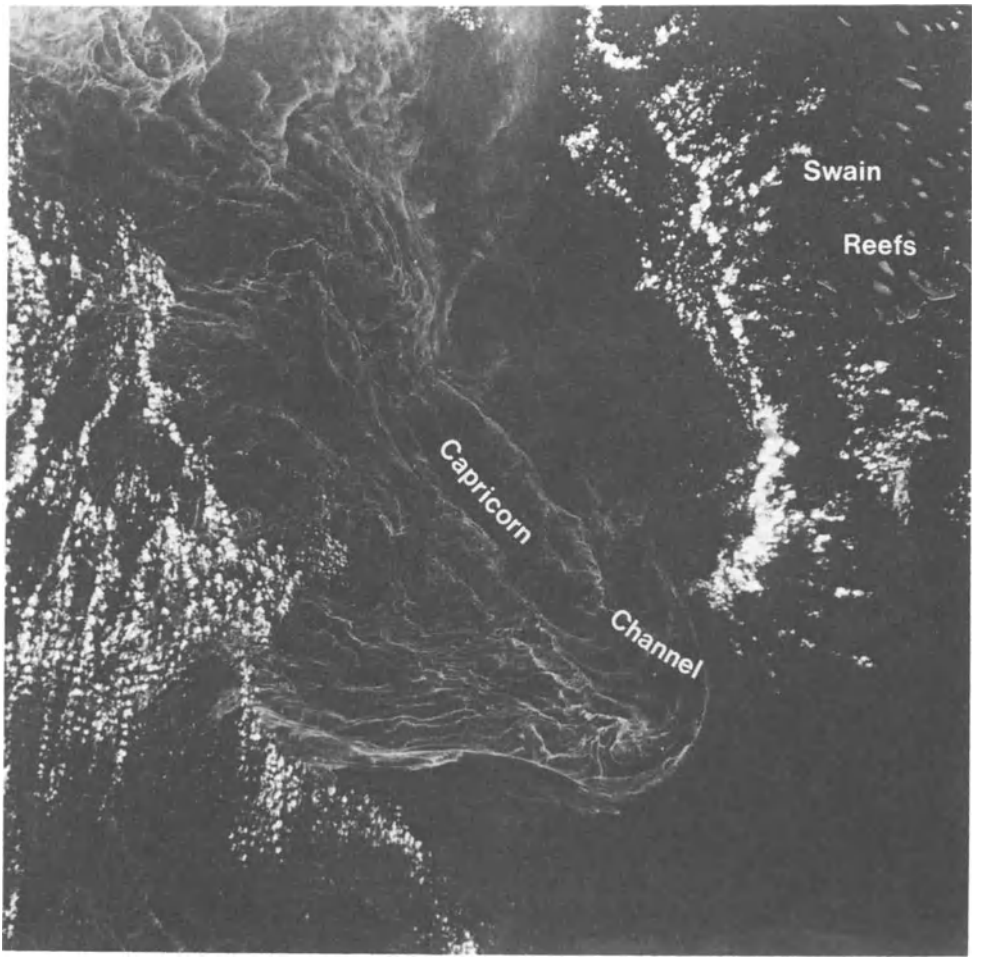

Figure 3. Surface aggregations of *Trichodesmium* in the southern GBR photographed from the space shuttle on 29 August, 1983 (STS-09, Roll 35, Frame 1622).

To date, only two time series of data exist for the GBR which incorporate quatitative estimates of *Trichodesmium* abundances over annual cycles: the pioneering survey by Marshall (1933) at sites near Low Isles (16.5 S: Figure 2) and the two year survey by Revelante and Gilmartin (1982) near Townsville (ca. 19 S) The occurrance of *Trichodesmium* at both sites was highly episodic, lacking a clearly defined seasonal pattern. No obvious association was seen in either data set between *Trichodesmium* aggregations and nutrient input events: wind-driven sediment resuspension, floods, shelfbreak upwelling. Rather, blooms typically occurred during periods of calm weather. Once congregated at the surface, however, the distribution of aggregations was readily influenced by wind, particularly near the coast. Bloom events were of short duration and surface manifestations were observed to be highly aggregated (Marshall, 1933). Concentrations of *Trichodesmium* filaments were observed to reach $4x10^4$ per liter off Townsville (Revelante and Gilmartin, 1982). Taking 50 cells per trichome as a conservative estimate, estimated cellular numbers during bloom event peaks ($6x10^5$ - $2x10^6$ cells l^{-1}) can readily exceed abundances of other microplankton-sized phytoplankton taxa in GBR waters (Marshall, 1933; Revelante and Gilmartin, 1982; Furnas and Mitchell, 1986, Furnas unpubl.).

Phytoplankton standing crops in GBR shelf waters are modest in comparison to those observed elsewhere in temperate and polar ecosystems (Feldman et al., 1989). Given this, surface blooms of *Trichodesmium* episodically cover large areas within the GBR system. Wood (1965) observed surface blooms and aggregations along 1600 km of the Queensland coast and estimated the area affected to exceed $5x10^4$ km^2, approximately 20 percent of the total area of the NE Queensland shelf. Wolanski et al. (1984) reported that surface blooms of *Trichodesmium* (*Trichodesmium*) complicated the interpretation of island wakes in LANDSAT imagery of the Torres Strait. The surface features were attributed to *Trichodesmium* on the basis of their (unspecified) spectral signature. The most dramatic observation of a massive *Trichodesmium* bloom within the GBR system was made using hand-held photography during a 1983 NASA space shuttle mission (Kuchler and Jupp, 1988). In this case, a surface bloom of *Trichodesmium* extending over 150 km in length was observed within the Capricorn Channel (22-23 S) at the southern end of the GBR (Figure 3). The nature of the bloom was confirmed by ground (seagoing) observers. Similar blooms have subsequently been retrospectively identified in LANDSAT MSS (Band 4) imagery (D. Kuchler, pers. comm.). The interpretation of remotely sensed imagery for indications of *Trichodesmium* aggregations at the surface may at times be complicated by the presence of surface slicks of coral gametes and larvae following mass spawning events (Oliver and Willis, 1987). Spawning in corals follows a lunar cycle, but is seasonally most intense during the austral summer which coincides with the time of year when *Trichodesmium* aggregations occur most frequently. While coral spawn and *Trichodesmium* may co-occur within a slick, slicks of different origin should show differing spectral signatures.

At this time, mechanisms responsible for the development of episodic *Trichodesmium* blooms in the GBR, particularly those of massive scale, are unresolved. The fact that surface blooms can appear during periods of calm weather clearly points to the role of buoyancy in bringing populations otherwise dispersed by vertical mixing to the surface. At most times and locations, vertical density gradients in GBR shelf waters are small and ephemeral. Once aggregated at the surface, *Trichodesmium* may be concentrated by winds, Langmuir cells and tidal currents. The lack of quantitative data on the size and dynamics of *Trichodesmium* populations dispersed through the water column on the shelf scale precludes estimation of whether surface aggregations represent the outcome of real population growth within a region, or merely the concentration of a population dispersed through the water column.

Whatever the nature of their formation, *Trichodesmium* aggregations exert direct and indirect effects upon natural populations of other phytoplankton in the GBR. Changes in both the abundance and diversity of diatom populations (Figure 4) can be noted to coincide with *Trichodesmium* blooms (Marshall, 1933; Revelante and Gilmartin, 1982; Revelante et al., 1982).

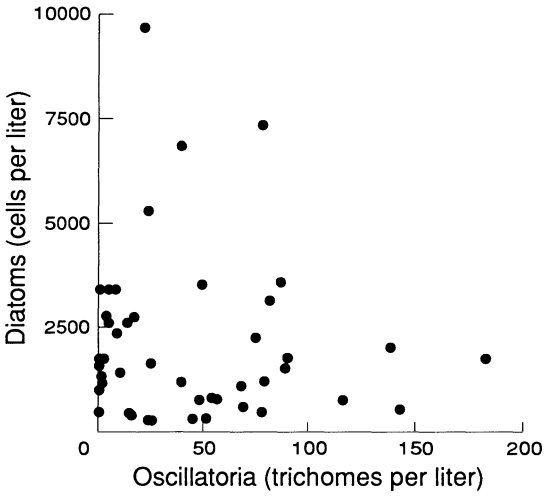

Figure 4. Relationship between depth-weighted mean water column abundances of diatom cells and *Trichodesmium* filaments near Low Isles, July 1928 - July 1929. Data were plotted from Marshall (1933).

Biomass (chlorophyll) specific production rates declined during *Trichodesmium* aggregation events, though absolute production rates were high due to the high standing crops. Revelante and Gilmartin speculated that materials released by the *Trichodesmium* were inhibitory to local diatom populations.

In other studies, Jones et al. (1986) found that changes in the speciation of iron and cadumium occurred during *Trichodesmium* blooms and could be simulated with fresh (non-photolyzed) extracts from *Trichodesmium*. These experiments did not resolve whether the interactions between *Trichodesmium* and diatoms were due to direct inhibition, or through indirect modifications to trace metal or other elemental availability.

2. SUMMARY

Pelagic *Trichodesmium* is a very visible element of the plankton of the GBR shelf ecosystem. Efforts to understand the role of *Trichodesmium* within the GBR ecosystem are currently constrained by the lack of quantitative information on the *in situ* abundance and spatial and temporal variability of *Trichodesmium* populations, particularly when they are dispersed through the water column.

3. ACKNOWLEDGEMENTS

The satellite photograph was made available by the U.S. Department of the Interior, U.S. Geological Survey EROS Data Center. Dr. D. Kuchler (Mapping and Monitoring Technology Pty. Ltd., Townsville, Qld.) kindly made LANDSAT MSS imagery available for inspection. This is AIMS contribution no. 553.

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EFFECT OF TRICHODESMIUM BLOOMS ON WATER QUALITY IN THE GREAT BARRIER REEF LAGOON

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ABSTRACT. Studies carried out in Cleveland Bay and John Brewer Reef in the central Great Barrier Reef have shown significant changes in water quality during the presence of *Trichodesmium* blooms. Elements most affected include particulate Fe and Mn, dissolved Mn and Ni, as well as pH, suspended sediments, phosphate, nitrate, chlorophyll-a and ammonium. Speciation measurements have established that Cd, Pb and Cu ions in seawater become more bioavailable during the presence of *Trichodesmium*. Coincident with the increased bioavailability of trace elements during the blooms, significant and sustained increases in total Fe, Zn, Cd, Cu, Ag and Mn occurred in the black lip oyster, *Saccostrea amassa*. Levels of Zn, Cd, Cu in S. *amassa* exceeded health guidelines by 8000%, 4000% and 3000% respectively. Increased bioavailability of some trace elements during *Trichodesmium* blooms may also be a cofactor along with anthropogenic input for the increased metals in these oysters.

1. Introduction

Increases in nutrient levels in waters of the central Great Barrier Reef Lagoon (GBRL) have recently caused considerable concern over possible algal displacement of corals and even destruction of coral reefs (Bell and Gabric, 1990; GBRMPA, 1988; Rasmussen and Cuff, 1990). Soil erosion accelerated by land clearing and overgrazing of livestock are probably the most serious contributors to nutrient loading (Walker, 1991). Agricultural fertilizers and sewage discharge have also been implicated (GBRMPA, 1988). However, to date there have been no firm quantitative data which demonstrate that nutrient levels for large areas of the reef are rising (Walker, 1991). It is surprising that few investigators draw the corollary between algal-induced trace element changes reported in earlier years (Jones *et al.*, 1982; Jones *et al.*, 1986; Jones and Thomas, 1988) with recent concerns over algal-related nutrient levels in reef waters. This communication reviews some of the earlier information which shows that during *Trichodesmium* blooms in the GBRL significant changes in water quality of both dissolved and particulate trace elements occur. Of concern is the marked increase in the labile or toxic fractions of Cd, Pb and Cu during the senescent phase of *Trichodesmium*. Coincident with increases in labile trace elements in the water column, marked increases in trace elements occurred in the oyster *Saccostrea amassa*. Recent results of nutrient studies carried out at John Brewer Reef 70 km

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offshore from Townsville have also shown significant changes in nutrient concentrations during *Trichodesmium* blooms.

2. Area of Study

Studies were carried out in Cleveland Bay, Townsville, and offshore at John Brewer Reef on the tropical north east coast of Queensland, Australia within the central region of the GBRL (Figure 1). The Great Barrier Reef (GBR) lies approximately 65 km offshore at Townsville, but many fringing reefs are present in the GBRL, the region between the mainland and the GBR, where mean depths are close to 35 m (Pickard et al., 1977).

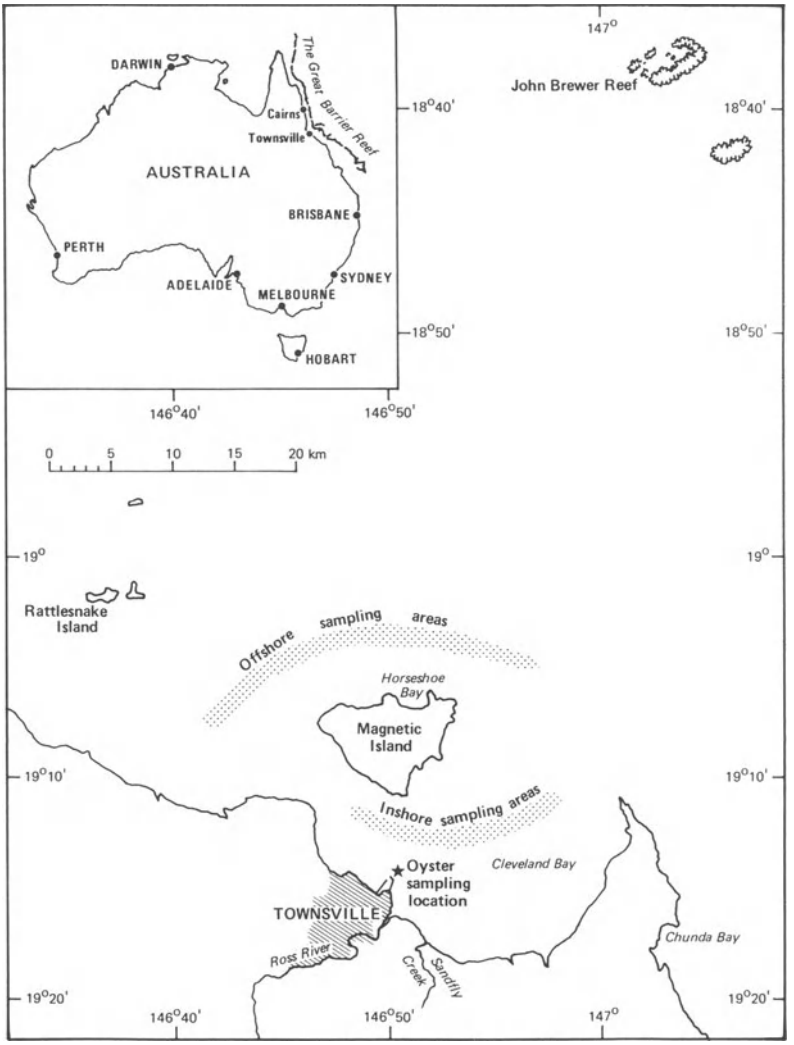

Figure 1. Study area.

The climatic conditions define two main seasons (Revelante and Gilmartin, 1982). A season of high precipitation associated with the north westerly monsoon (December-April), characterized by high temperatures, low salinities, low densities, some degree of increased vertical stability and a season of low to no precipitation (May-November) associated with the SE trade winds, characterized by low temperatures, high salinities, high densities and a vertically well mixed water column. Calm periods occur predominantly during this time and are often accompanied by prolific *Trichodesmium* blooms (Jones, 1981; Revelante and Gilmartin, 1982).

As the coastline at Townsville is a SE sloping coastline, for much of the year *Trichodesmium* blooms would travel parallel to the coast. However, during November-December a 45 year wind analysis of our region (Townsville Meteorological Office) shows that predominant winds are NE, both in the morning and afternoon, thus concentrating *Trichodesmium* blooms inshore. During this period tidal amplitude is increasing, consequently significant amounts of the blooms affect inshore and estuarine regions (Jones, 1981; Jones *et al,* 1982). For these reasons studies have been carried out on inshore and offshore waters during *Trichodesmium* blooms in Cleveland Bay. The seaward side of the harbour breakwater was selected for a detailed study (Figure 1) of the effects of *Trichodesmium* on trace element cycling in the water column (Jones *et al.,* 1982) and in the oyster *Saccostrea amassa.* This well mixed location is exposed to *Trichodesmium* from the onshore movement of surface water. In addition more recent studies have been carried out at John Brewer Reef, 70 km off the coast of Townsville.

3. Distribution of *Trichodesmium*

3.1. AUSTRALIAN COASTAL WATERS

Prolific blooms of *Trichodesmium* occur in the GBRL (Jones, 1981; Revelante and Gilmartin, 1982). On some occasions blooms can extend for many hundreds of square kilometres along the Queensland coast, extending from the shoreline to the outer barrier reef and occupying tens of thousands of square kilometres of sea surface (Jones *et al.,* 1982; Kuchler and Arnold, 1986). *Trichodesmium is* also prolific off the west coast of Australia in the Dampier Archipelago, and occurs at different times of the year from Rottnest Island, off Perth to the North West Shelf. The blooms are very common in Mermaid Sound in the summer months and in Noname Bay, Dampier Archipelago, where fish kills occasionally occur (Creagh, 1985; Simpson (EPA, Perth), personal communication).

The hypotheses concerning the cause of the cyclical appearance and disappearance of *Trichodesmium* have often been discussed (Fogg; 1982; Borstad, 1982), but no clear reason for their formation has yet been presented. Carpenter and Price (1976) have suggested that as nitrogen fixation by this alga is adversely affect by turbulence, this is one reason why *Trichodesmium* blooms usually occur in calm and sunny conditions. However in Townsville's coastal waters *Trichodesmium* blooms often occur more abundantly in the September-December period every year, and often occur during calm weather after turbulence. For more details on phytoplankton abundance and variation in the GBRL, the reader should consult Furnas and Mitchel (1986).

3.2. CLEVELAND BAY

Phytoplankton species have been monitored in surface waters of Cleveland Bay from 1975-1979 (Walker, unpublished results), along with the water quality investigations. Weekly examinations revealed a flora dominated by diatoms and the blue-green alga *Trichodesmium.* Pennate diatoms normally outnumbered centrics. This was largely due to the abundance of *Cylindrotheca closterium* and to a lesser extent *Navicula* species. These two species and other small pennates appeared to be the major components of the nanoplankton. Walker (unpublished results) found that the important genera of the net plankton were *Chaetoceros* and *Rhizosolenia.* Dinoflagellates, although always conspicuous, were rarely a dominant group. The organism that occurred in the greatest numbers was *Trichodesmium.*

Two species of *Trichodesmium* occur in Townsville's coastal water, *Trichodesmium erythraeum* (Ehrenberg) and *Trichodesmium thiebautii* (Gomont) (Revelente, personal communication). T. *erythraeum* seems to be the more abundant species inshore, and occurs as aggregated rafts or trichomes, and as filaments which are interwoven to form a "ball-like" structure (Taylor *et al.,* 1973). These filaments float on, or just below the surface of the sea forming large, orange-brown slicks which are distinctly visible from the air (Jones, 1981).

The term "red-tide" for *Trichodesmium* blooms may be misleading as this term can imply adverse effects such as massive fish kills and shellfish poisonings (Dale and Yentsch, 1978). In these cases the toxic dinoflagellates, *Gonyaulax sp., Gymodinium* sp. and *Pyrodinium sp.* have been indicated as the causative organisms. Blooms of T. *erythraeum* are generally regarded as non-toxic (Fogg *et al.,* 1973), although local knowledge suggests that it is not advisable to eat certain species of fish in October. Toxins have recently been identified in *Trichodesmium* from the Caribbean (see Hawser and Codd in this volume).

4. Methods

Procedures used for trace element analysis of waters and suspended sediments have been detailed in previous publications (Jones *et al.,* 1982; Jones and Thomas, 1988). Concentrations of $P0_4$, $N0_2$, $N0_3$, NH_3, Si were determined by using an autoanalyser technique (Ryle *et al.,* 1981).

Oysters (24), were collected monthly from the seaward side of the eastern harbour breakwater (Figure 1), and were placed in a plastic bucket containing seawater collected from that station. The oysters were evacuated of suspended sediments, faecal material etc., in the laboratory by aeration with a bubble stone for two days. The organisms were frozen at -20°C. The length of each oyster was measured with calipers, and grouped into 4 batches of six oysters (mean shell length 5.3 +0.2 cms). Although separated tissues (adductor, mantle, gills and palps, kidney, visceral mass) were analysed (Jones, 1981), the results reported here are for the whole organism. Separated tissues were subsequently dried to a constant weight in an oven at 65°C, and wet weight/dry weight measurements made on each composite sample. Digested tissues (HN03/HC104) were analysed for Fe, Mn, Zn, Cu, Ni, Cd, Ag by atomic absorption spectrophotometry with background correction.

5. Effect of *Trichodesmium* Blooms on Water Quality

5.1 CLEVELAND BAY

In the Cleveland Bay study *Trichodesmium* blooms (Figure 2a) were predominantly concentrated inshore (mean 8515 ±4700 trichomes liter^{-1}), compared to offshore stations (mean 3300 ±4000 trichomes liter^{-1}). This was due to the prevailing onshore winds and the buoyant nature of *Trichodesmium* trichomes. Consequently higher levels of seston (suspended sediments) were evident in inshore waters (15.3 ±13 mg liter^{-1}) during the blooms (Figure 2a) compared to offshore waters (7.8 ±5 mg liter^{-1}). Inshore stations were characterised by much higher levels of particulate Fe, Mn and Cu than offshore waters (Figure 2ab). The difference for Fe and Mn was highly significant ($P < 0.005$). Inshore/offshore comparisons for dissolved trace elements were not significant for most elements, with the exception of Fe (Figure 2c). Iron was much higher in offshore waters (1.4 +1.5 µg liter^{-1}) than inshore waters (0.8 + 0.5 µg/l). Significant differences in particulate: dissolved element ratios were also evident for most elements. For example, the mean particulate iron:dissolved iron ratio was 306:1 (inshore) and 17:1 (offshore). Ratios for other elements were Mn (14:1 inshore, 6:1 offshore), Zn (1.7:1 both cases), Cu (5.7:1, 1.4:1), Ni (0.9:1, 0.5:1).

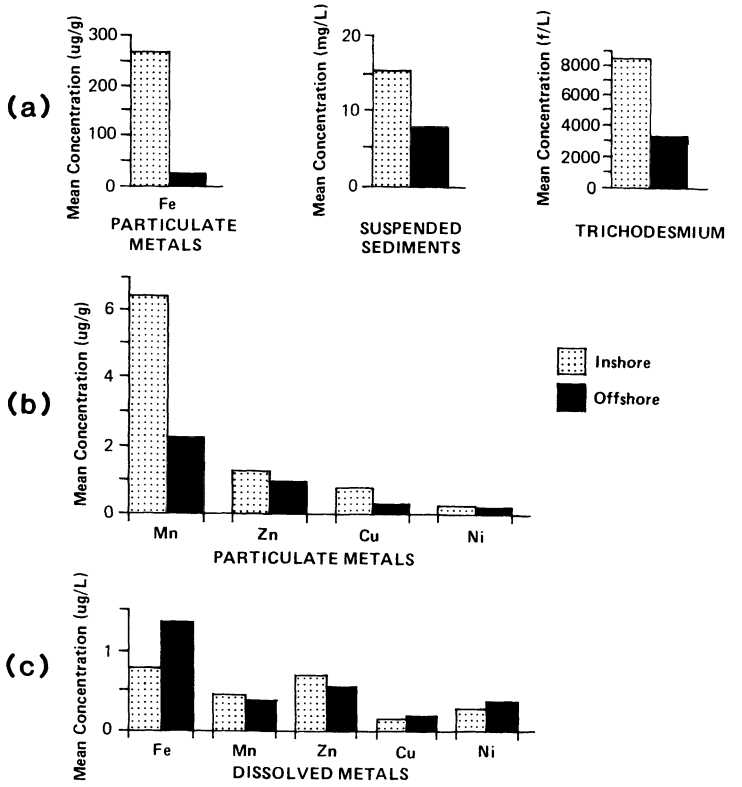

Figure 2. Inshore/offshore comparisons of (a) particulate Fe, suspended sediments, *Trichodesmium*, (b) particulate, and (c) dissolved metals in Cleveland Bay.

5.2. HARBOUR BREAKWATER (SEAWARD SIDE)

Results of *Trichodesmium* counts, humic acids, suspended material, salinity, rainfall and temperature (Figure 3) showed that the main pulse of *Trichodesmium* trichomes was detected in the water column from late August to mid October when temperatures were low (23-27°C), salinities were high (36%), and rainfall was negligible (<30 mm). In addition the presence or absence of senescent trichomes (see hatched bar in Figure 3) was also assessed. High densities of senescent trichomes often occurred after *Trichodesmium* blooms, particularly from October to January when suspended material was high and very variable. Humic acid concentrations often peaked during such periods (Figure 3). Salinity and rainfall results suggested that these humic substances were marine and were not land derived.

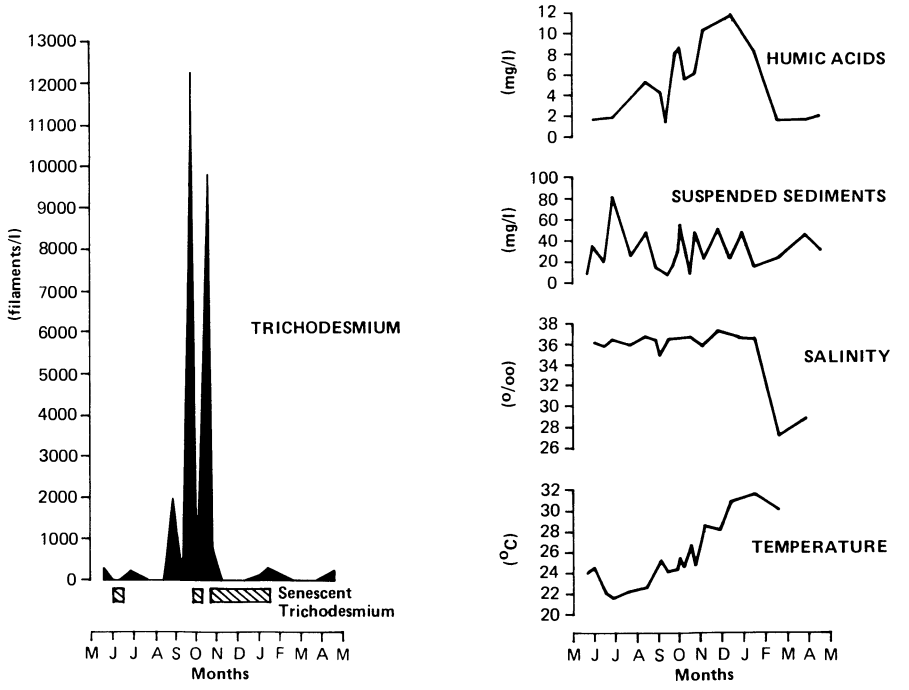

Figure 3. *Trichodesmium* and related water quality parameters at the harbour breakwater from May 1978-April 1979 (hatched bar represents senescent *Trichodesmium).* [Reproduced from Jones *et al.*, 1982 with permission from *Oceanologica Acta.]*

During the twelve month study at the close inshore location (Figure 1) dissolved iron was the only element that was higher in concentration in the wet season (period 4, February/March) than

the other periods (Figure 4a). Levels of dissolved Mn were significantly higher ($P < 0.005$) in the senescent period (period 3, November to mid January), than all the other periods, and dissolved Ni was significantly higher during the bloom and senescent periods (periods 2 and 3, late August-mid January). The most significant finding was that dissolved Mn, Zn, Cu, and Ni concentrations were high throughout most of the investigation. Although particulate Zn and Ni concentrations (Figure 4b) did not vary markedly, particulate Fe, Mn and Cu generally increased during the transition from bloom to senescent to monsoonal periods (periods 2-4). The increases in dissolved and particulate trace elements during the presence of *Trichodesmium* lasted 10-12 weeks prior to the wet season.

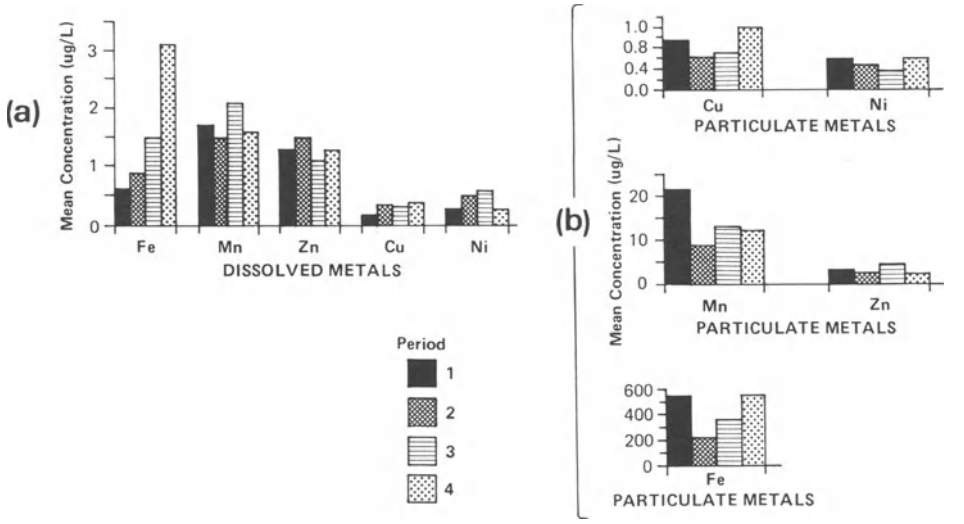

Figure 4. Mean (a) dissolved and (b) particulate trace element variation at the harbour breakwater. Period 1 Pre-bloom; Period *2 Trichodesmium* bloom; Period 3 Senescent bloom; Period 4 Wet season.

Speciation measurements using differential pulse anodic stripping voltammetry (DPASV) showed that the labile fractions of Cd, Pb, Cu increased during the transition from no *Trichodesmium* (period 1) to bloom (period *2),* to the senescent period (period 3) (Figure 5). More importantly the labile fractions of all 3 metals were higher than the labile fractions determined solely in the wet season when all labile Cd, Pb, Cu fractions were close to 40% of the total dissolved fractions (Cd 40%, Pb 44%, Cu 40%). This has particular significance, since labile fractions of metals measured by the DPASV technique are equivalent to the toxic, or more bioavailable fractions (Florence, 1989).

6. Metal Variation in the Oyster *Saccostrea amassa*

Most separated tissues of *S. amassa* increased significantly in dry weight during *Trichodesmium* blooms (Figure 6) with the gills and labial palps showing the most distinct increases. During August-December, when algal counts were highest, purging of oysters released considerable quantities of *Trichodesmium.* Although these oysters would be feeding on other forms of phyto-

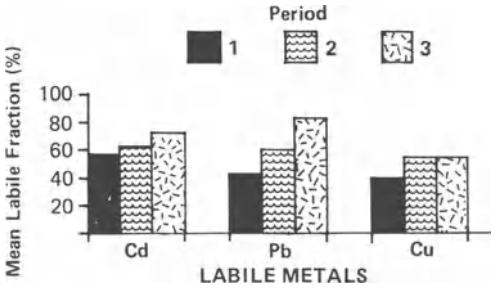

Figure 5. Mean labile trace element variation at the harbour breakwater. Period 1 No *Trichodesmium;* Period 2 *Trichodesmium* bloom; Period 3 Senescent *Trichodesmium*

plankton, microscopic counting of all the years water samples, that had been preserved, revealed that for 83% of the year *Trichodesmium* and/or senescent trichomes were detected in the water column. Good inter-tissue growth correlations ($P < 0.005$) occurred between the mantle tissue and the gills and palps, visceral mass, and muscle.. A highly significant correlation between the dry weight of the mantle tissue and *Trichodesmium* filaments (correlation coefficient 0.70 $P < 0.005$) was evident. Observations on the shell growth of the cultivated oyster S. *echinata* by K. Bryson of Horseshoe Bay, Magnetic Island have established that the growth of this oyster significantly increases when *Trichodesmium is* transported into Horseshoe Bay.

Total concentrations of trace elements in S. *amassa* are shown in Figure 7. Significant increases in total Zn, Cd, Cu occurred in the wet season of February-April (sample periods 16-17). Significant and sustained increases in Fe, Zn, Cd, Ag, Mn occurred during the *Trichodesmium* season from September-December (sample periods 10-16), the period within which S. *amassa* was sexually mature. The sustained increases in Mn are noteworthy. Manganese has been suggested as a trace element which is actively involved in the sexual cycle of the oyster (Galtsoff, 1942, 1964). This author found a similar seasonal cycle for Mn in the oyster *Ostrea virginica* collected from Long Island Sound, Connecticut (USA). Lowest values occurred in the winter (7-11 $\mu g\ g^{-1}$) with the highest values in the summer (28-57 $\mu g\ g^{-1}$). Marine algae were observed in the digestive tract of the oysters collected in the summer, but discounted as a factor in the

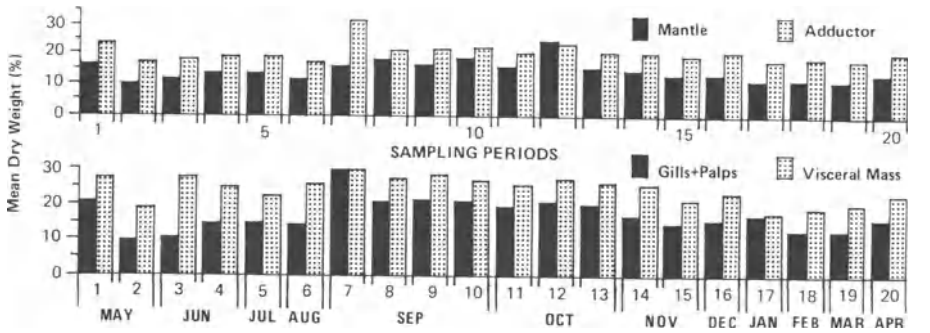

Figure 6. Variation of mean dry weight (%) in separated tissues of *Saccostrea amassa* at the harbour breakwater.

seasonal fluctuations of Mn in the oysters. Increases in manganese coincided with approximate periods of ripening of the gonads and spawning of the oysters. Galtsoff (1942) found that among various organs in 0. *virginica,* ripe ovaries were richest in Mn (51-60 mg kg^{-1}). Testes were low in Mn (4.6-7.3 mg kg^{-1}).

7. Comparison of metal levels in *S. amassa* with other regions

Comparison of mean trace element concentrations in whole tissues of S. *amassa* with guideline values set by the Australian National Health and Medical Research Council for metals in oysters (Table 1) showed that Zn, Cd, and Cu levels were exceeded by 8000% (Zn), 4000% (Cd), and 3000% (Cu). A comparison of Zn levels in *S.. amassa* from seven locations in tropical coastal

Table 1. Comparison of mean trace element concentrations in whole tissues of *Saccostrea amassa* with guideline values set by Australian National Health and Medical Research Council.

	Fe	Mn	Zn	Cu	Ni	Cd	Ag
Range	54-450	6-35	673-20906	508-3246	0.2-18.0	1-12	1-14
Mean ± Std Dev	200 ±104	18 ±9	8250 ±4800	1635 ±800	3.6 ±4.3	4.3 ±3.0	6.2 ±3.3
*	-	-	100	50	-	1	-

* Australian Health Standards for Metals in Oysters (μg/g)

waters of Australia (Table 2) indicated that oysters collected from the central GBR region are enriched in this metal. At Rattlesnake Island and Chunda Bay (Figure l) Zn in oysters exceed the recommended health levels by 1200%. These locations are remote from industrial sources of these elements.

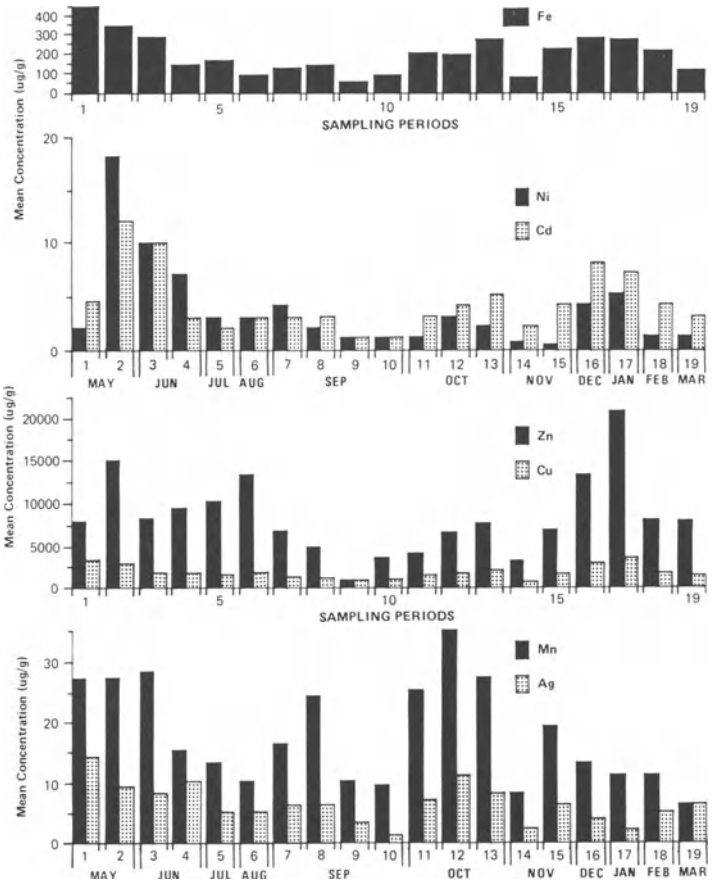

Figure 7. Variation of mean total trace elements (llg/g) in *Saccostrea amassa* at the harbour breakwater.

The mean concentrations of zinc in S. *amassa* from Cleveland Bay are comparable to levels of zinc in oysters from the polluted Tamar Estuary in Tasmania (Ayling, 1974). The upper range of Zn in oysters from the harbour breakwater was close to the highest ever recorded concentration of zinc in oysters (38,700 ppm d.w.) reported for the Derwent Estuary, Tasmania, one of the most polluted areas of the world (Bloom and Ayling, 1977). Copper levels were even higher than

reported for the Tamar Estuary (Ayling, 1974). In contrast, nickel concentrations in oysters were comparable to concentrations reported in many species of oysters from various regions of the world (Forstner and Wittmann, 1981), supporting the view that the bioconcentration of this element is usually quite low. Our studies have demonstrated that increases in metals do occur in oysters during the *Trichodesmium* season and that at this location Zn and Cu concentrations are extremely high. In addition, concentrations of zinc in oysters collected from the Hook Island Observatory and close to zinc anodes and sewage discharge reflected the ability of S. *accostrea* to pick up zinc contamination (Table 2), supporting the view that this is a good monitoring organism.

Table 2. Zinc levels in *Saccostrea* species at seven locations in tropical coastal waters of Australia.

Site	Level (μg/g)	Reference
Townsville Harbour (seaward station)	Range: 673-20906 Mean (N = 19): 8253	Jones (1981)
Rattlesnake Island	Range: 1441-2095 (N = 12): 1723	Burdon-Jones, Denton, Jones & Mean McPhie (1976)
Chunda Bay	Range: 1443-2452 Mean (N = 12): 1701	Burdon-Jones, Denton, Jones & McPhie (1976)
Darwin Harbour	Range: 109-611	Peerzada and Dickinson (1988)
Dampier Archipelago	Range: 360-1800	Talbot (1985)
Shark Bay (W.A.)	Mean (N = 7): 180	McConchie *et al.* (1988)
Hook Island Observatory		
proximal to observatory	Range: 1225-9648 Mean (N = 40): 2864	Stump (1988)
distant from observatory	Range: 131-1314 Mean (N = 20): 500	

8. Variation in nutrient water quality at John Brewer Reef

Results reported in Jones *et al.*, 1989 summarises a thirteen month water quality investigation carried out at John Brewer Reef, 70 km off the coast of Townsville (Figure 1). This work was done from 1988-89 in order to ascertain the effect of the world's first floating hotel on the water quality of the lagoon (Jones *et al.*, 1989). No marked differences in mean concentrations of phosphate, nitrate, chlorophyll-a and suspended sediments were detected at either of the control and hotel station during the presence or absence of the hotel (Jones et *al.*, 1989). However, when the water quality of the lagoon was assessed during the presence or absence of the filamentous

blue-green alga *Trichodesmium*, marked changes in the nutrient concentrations were evident (Table 3).

Table 3. Mean nutrient concentrations at John Brewer Reef during the presence and absence of *Trichodesmium*.

	Ch 'a' (µg/l)	PO_4 (µM)	NO_3 (µM)	NH_3 (µM)	Susp Sed (mg/l)	pH
Absence of Trichodesmium						
	0.49(70)	0.19(83)	0.22(79)	0.22(36)	2.58(63)	8.27(40)
±SD	0.58	0.07	0.12	0.10	0.91	0.04
Presence of Trichodesmium						
	0.32(40)	0.26(50)	0.32(45)	0.09(25)	4.08(38)	8.42(28)
±SD	0.23	0.06	0.14	0.02	0.98	0.04
% Change During Blooms	35% ↓	37% ↑	55% ↑	59% ↓	56% ↑	ΔpH = 0.15
Derived Coral Maximum Tolerance Levels for 20% decrease of growth	0.59	0.25	1.31	0.65	3.85	- Hawker & Connell (1989)

Number of determinations in parenthesis.

Increases in pH (0.15 pH units), suspended sediments (56%), nitrate (55%) occurred in the lagoon during the presence of *Trichodesmium*, compared with concentrations in the lagoon when this alga was absent from the water column. Chlorophyll-a and ammonium decreased 35% and 59% respectively when *Trichodesmium* was present, compared to previous concentrations when the alga was absent from John Brewer Reef. These anomalous changes in nutrients, suspended sediments, chlorophyll-a and pH occurred at both the hotel and control stations at the start of summer in November-December 1988. Suspended sediments and phosphate levels exceeded or were close to recent tolerance levels which would cause a 20% decrease in coral growth (Hawker

and Connell, 1989). Some investigators have suggested that *Trichodesmium* has an inhibitory effect on the growth of phytoplankton (Revelante and Gilmartin, 1982). The decrease in chlorophyll-a during the presence of this alga and the negative correlations of chlorophyll-a with PO_4, NO_3, NH_4 (-0.21 to -0.27) tend to substantiate these earlier observations. This is the first documented case of this phytoplankter affecting nutrient concentrations at an offshore reef system, distant from land based activities. The marked decrease in NH_4 concentrations from 0.22 µM (*Trichodesmium* absent) to 0.09 µM (*Trichodesmium* present) could suggest that this alga assimilates NH_4-N in preference to other forms of nitrogen in seawater. Consequently this organism may bloom more frequently in regions significantly affected by man-made nitrogenous inputs of nutrients such as sewage discharge, as occured in Cleveland Bay (Jones and Tirendi, 1988). This is certainly suggested for the harbour breakwater station where *Trichodesmium* and/or its decomposition products occurred in the water column for most of the 12 month study.

9. Conclusion

The water quality studies to date in Cleveland Bay suggest that *Trichodesmium* blooms are the most dominant phytoplankter in the bay for much of the year. It is believed that this is due to the increased flux of nutrients to this bay from the discharge of about 20 million litres of raw sewage per day from 1975 to 1987. Although sewage is now partially treated, some 30 million litres day^{-1} enter the bay at Sandfly Creek contributing about 58 tonnes of excess P and 219 tonnes of N per year, plus associated trace metals (Jones and Tirendi, 1988). Increased concentrations of several trace elements occur in the water column during *Trichodesmium* blooms, with particulate fractions of Fe and Mn being the most significant. Significant increases in suspended sediments occur during the blooms (Cleveland Bay, Harbour Breakwater, John Brewer Reef). Of particular concern is the marked increase in the labile fractions (the bioavailable fraction) of Cd, Pb and Cu during extended *Trichodesmium* blooms. The enrichment of Zn, Cd, Cu in S. *amassa* to levels which exceeded the NHMRC guidelines for seafood could reflect the proximity to metal (Cu, Zn, Pb) shipments through the Port. However sewage discharge cannot be discounted since raw sewage discharge at the mouth of the Ross Estuary (Figure 1) only stopped in 1987 when it was transferred to the Sandfly Creek outlet. In addition the conversion of particulate/colloidal fractions of metals to labile fractions during extended *Trichodesmium* blooms (Jones *et al.*, 1982) may also be a cofactor.

The studies to date have therefore demonstrated that *Trichodesmium* blooms significantly affect trace metals in close inshore waters, whilst the nutrient studies at John Brewer Reef demonstrate that such effects may be widespread. As *Trichodesmium* blooms are so prolific in the GBR it is believed that significant environmental effects will be observed in those regions of the GBRL which experience extended bloom events of this alga. There is therefore an urgent need to integrate nutrient and trace element studies in the GBRL.

10. Acknowledgements

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IRON NUTRITION OF *TRICHODESMIUM*

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ABSTRACT. The diazotrophic metabolism that allows *Trichodesmium* to thrive in nitrogen-poor oligotrophic oceans relies on many iron-containing enzymes and proteins. The iron dependent processes in *Trichodesmium* and the theoretical amount of iron required for these processes are comparable to the measured iron content of colonies from the open ocean. The high iron requirement of these species is problematic in light of the limited iron delivery to open ocean systems. *Trichodesmium* can take up soluble forms of iron and has unique mechanisms that may allow it to use iron contained in dust particles. Their ability to add new nitrogen through nitrogen fixation and their potential for increasing the amount of biologically available iron make *Trichodesmium* crucial organisms in the geochemistry of tropical and sub-tropical oceans.

1. Introduction

In tropical and sub-tropical oceans the predominate nitrogen-fixer is the marine filamentous cyanobacterium *Trichodesmium*. Open ocean nitrogen fixation has been hypothesized to be constrained by many factors including turbulent breakup of *Trichodesmium* colony integrity (Carpenter and Price 1976), phosphate limitation (Doremus 1982), sulfate inhibition of molybdate uptake (Howarth et al. 1988) and copper toxicity (Rueter et al. 1979). The case has also been made for iron control over nitrogen fixation (Rueter 1983) based on the high iron requirement for nitrogen fixation in relationship to the low availability of iron in surface ocean waters. Iron is required in many metabolic processes including photosynthesis, respiration, nitrogen metabolism and oxygen protection, but

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nitrogen fixation metabolism requires that all of these processes be energy efficient and, in addition, supply enough ATP and reductant to reduce N_2 to ammonium. Relative iron requirements for growth on N_2 versus NO_3^- were used to partition natural waters (oceans and lakes) based on the ratio of the flux of biologically available iron to the flux of combined nitrogen sources (primarily nitrate, ammonium, or urea) into three types; one region would have enough iron but low combined nitrogen, and nitrogen fixation would be feasible. The second region would have too little iron to effectively fix nitrogen, and the third region would have low iron and high combined nitrogen such that iron efficient cells would be favored. Tropical and sub-tropical regions have been classically described as being nitrogen limited due to the limited availability of nitrate; however, because nitrogen fixation is also limited in these regions, they should also be considered iron-limited. This means that in these waters there is sufficient iron to support growth on nitrate or ammonium but not enough to cause the community to change to organisms that fix dinitrogen.

In the open oceans, the biological availability of iron is determined by the wind-borne transport of particles (aeolian dust). Only a limited fraction of the iron that is contained in this fine-grained continental material is soluble, estimated to range from 10% (Moore, Milley, and Chatt 1984) to up to 50% in some cases (Zhuang et al. 1990). The current consensus is that these dust particles fall on the ocean surface and only the iron that dissolves is taken up by phytoplankton (Rich and Morel 1990). The undissolved iron sinks out of the water column, and even the soluble, or "biologically available" iron taken up by phytoplankton eventually sinks or is transported out of the surface water. In deep waters the concentration of soluble, or biologically available, iron is low, and this may be due to either efficient biological uptake or chemical precipitation and scavenging or both. This is in contrast to the other nutrients for phytoplankton growth, nitrate and phosphate, which are soluble and in high concentrations in deep water. Thus when deep water is advected to the surface it is low in biologically usable iron but high in other nutrients. This explains why surface phytoplankton growth in open ocean systems may depend on aeolian dust for up to 95% of their iron (Duce 1986). It is paradoxical that if the oceans are really iron limited as suggested (Martin 1990, Martin et al. 1990) so much of the iron slips through the system without being acquired by organisms. It is very possible that *Trichodesmium* may play a much more active role in the interception, suspension and leaching of the iron from dust particles than has been observed for other phytoplankton.

Experimental results on the effect of iron on nitrogen fixation and growth of *Trichodesmium* have been presented previously (Ohki et al. 1991; Rueter et al. 1990; Rueter 1988). This paper addresses the more general hypothesis that portions of the open oceans are simultaneously limited by nitrogen and iron availability and that *Trichodesmium* species play crucial roles by providing both new nitrogen and iron to the community. The new nitrogen is that resulting from N_2 fixation, and this rate process depends on the light and iron available. The new iron is obtained by interception and leaching of particles that might have otherwise sunk quickly through the water column. *Trichodesmium* may also help keep iron in surface waters through its buoyancy and resistance to predation. Thus, *Trichodesmium* may be considered a critical organism geochemically (a "COG") in the cycles of nitrogen and iron open ocean communities, and the particular details of how *Trichodesmium* is able to balance the requirements for iron and nitrogen may be a major factor in determining the nature of biological productivity in oligotrophic oceans.

2. Effects of Iron on Physiology of *Trichodesmium*

Iron nutrition is essential for the photosynthesis and nitrogen fixation in *Trichodesmium*. Additions of iron to natural samples of *Trichodesmium* collected in the oligotrophic waters near Barbados resulted in stimulation of carbon fixation and nitrogen fixation rates (Rueter 1988). The changes in these rates were accompanied by increased chlorophyll-a and by an increase in the cellular fluorescence capacity (CFC). The CFC is an indication of the efficiency of energy transfer within the photosynthetic membranes, and increased CFC has been related to overall carbon fixation efficiency in the green alga *Scenedesmus quadricauda* (Rueter and Ades 1987). These responses of natural populations to iron are all consistent with the hypothesis that they are generally iron limited in the open ocean but respond to increases in iron supplied from the sporadic input of dust from the Sahara desert. This was tested by sampling *Trichodesmium* populations over a three month period from January through March in 1988 in the waters off the coast of Barbados. The biochemical content of natural populations of *Trichodesmium* varied after an episode of dust input in in a manner consistent with this hypothesis (Table 1); The chlorophyll-a , iron per colony and proportion of total protein as ferredoxin all increased. Suprisingly

Table 1. Iron dependent characteristics of natural populations of *Trichodesmium*. Data shown is for samples collected weekly between Feb. 1, 1988 and Feb. 22, 1988 from surface waters 5 km off of the coast of Barbados. There was visible transport of Saharan dust to the area on Feb. 7 and 8 as detected by the meteorological services at the Barbados airport. Chl-a was analyzed on 100 colonies by methanol extraction. Iron was measured on 50 colonies that were acid leached. The average values for these parameters was calculated from 3 months of weekly sampling data.

Parameter	Date 2/1	2/8	2/15	2/22	Ave.	n
Chl-a (pmol/col)	23	39	30	28	30	21
Fe (pmol/col)	---	140	110	90	140	16
Ferredoxin (ug/ug)	.0161	.0319	.0141	.0168	.0148	9
Flavodoxin (ug/ug)	.0020	.0020	.0009	.0012	.0008	9
Flavod./Feredox.	.12	.06	.06	.07	.05	9

the flavodoxincontent did not decrease, as has been shown in other iron limited cyanobacteria, when given iron (Jones 1988, Peschek 1979., Reith et al. 1986, Rogers 1987). Cultures of *Trichodesmium* NIBB 1067 grown with a range of iron demonstrated dependence of oxygen production and nitrogen fixation rates per chlorophyll-a on iron (Rueter et al. 1990), supporting the conclusions with natural samples. This work with cultures also demonstrated that iron-limited *Trichodesmium* were able to grow much better on NO_3^- than on N_2. These results indicate that the effect of iron limitation on *Trichodesmium* may be to impose a bioenergetic limitation on cell metabolism rather than

to limit any particular iron-dependent step. This means that nitrogen fixation requires a fully functioning photosynthetic apparatus to provide energy and carbon skeletons for nitrogen metabolism. The ability of *Trichodesmium* NIBB 1067 to fix nitrogen at ambient oxygen concentrations (Ohki and Fujita 1988) has been investigated at the molecular level and it has been hypothesized that the ability of these organisms to form inactive but oxygen protected forms of the nitrogenase Fe-protein may be crucial to maintain activity (Ohki et al. 1991). We have recently observed similar variation in the forms of the Fe-protein as a function of additions of iron (unpublished data) suggesting that iron nutrition may be involved in the ability of *Trichodesmium* to fix nitrogen in the presence of high oxygen concentrations. Thus, iron nutrition and iron-dependent processes are inseparable to the physiological competence of *Trichodesmium* to survive, and at times to thrive, in tropical and sub-tropical oceans around the world.

3. Iron and Nitrogen Content of *Trichodesmium*

If iron is limiting in the environment but the cellular content of iron is crucial to the overall metabolism, especially for the ability to fix nitrogen, the nitrogen content should be expected to be a function of the iron content. This relationship has been explored with other phytoplankton species in iron limited cultures. The results demonstrate that iron limitation affects the protein and pigment content, and that this results in protein nitrogen to iron ratios that are highest for the more severely iron limited cultures. For cultures of marine *Synechococcus* grown on nitrate, that ratio varied from around 600 up to 3000, meaning that the minimum amount of iron that the cells could live on was 1 iron atom for every 3000 nitrogen atoms (Rueter and Unsworth 1991). For the freshwater *Anabaena* growing on N_2 the average N:Fe ratio was 250 (Hutchins et al. 1991). This indicates that the iron content of *Anabaena* is higher than that of *Synechococcus* as would be expected because of the additional iron needed by *Anabaena* to fix N_2.

We hypothesized that the N:Fe ratio of *Trichodesmium* would indicate the metabolic state of the cells in the natural environment. To test this we measured the iron and protein nitrogen content in samples of natural populations of *Trichodesmium* collected in the Caribbean. Results are summarized in Table 2. Latitudes and longitudes of sampling stations where collections were made are shown for comparative purposes. Acid-leachable iron levels varied from 0.135 to 0.470 nmol colony^{-1} while the range in protein nitrogen content was between 51 and 204 nmol colony^{-1}. Colony Chl-a content was from 0.044 to 0.098 nmol colony^{-1} while nitrogen fixation rates ranged between 0.0054 and 0.6616 nmol N fixed colony^{-1} h^{-1}. These measured values show considerable variation due to the variable colony size as well as physiological parameters. Even the ratios of protein N to Fe had considerable variability, from a minimum of 166 (mol:mol) to a high of 707, with a mean value of 465. Molar Chl-a to Fe ratios were between 0.128 and 0.709. Although all samples collected during the day were fixing nitrogen, no direct relationship between measured iron content and nitrogen fixation rate could be ascertained. Protein nitrogen versus cellular iron content was plotted and all the data points can be fit into an envelope of ratios between 700 and 200 (Fig. 1). The upper boundary describes cells with very low iron levels (relative to cellular N, perhaps close to the minimum necessary for this organism to support nitrogen fixation metabolism. The minimum boundary, N:Fe around 200, may represent a storage limitation for iron in the cells. Iron

TABLE 2. Summary of iron and nitrogen measurements on *Trichodesmium* collected from the Caribbean. *Trichodesmium* was collected using gentle net tows and transferred for analysis by using a plastic inoculating loop. Iron measurements were made using the colorometric ferrozine technique (Stookey 1970, Gibbs 1979). Colonies (200) were place directly into 1 N HCl and leached overnight at 60 ° C. After leaching, the extracts were clarified on a desk top centrifuge. Total protein was measured using the BCA Protein Assay kit available from Pierce Chemical. Colonies were pipetted onto a Whatman GF/A filter, frozen and then ground in 1% SDS using a tissue homogenizer. This extract was centrifuged at top speed on a table top centrifuge before analysis. Protein nitrogen was calculated as before (Rueter and Unsworth 1991) based on the estimate that protein is 14% by weight nitrogen. Chl-a was measured with near-boiling methanol (Rueter et al 1990). Nitrogen fixation rates were measured by D. G. Capone and J. O'Neil and are presented here for comparison purposes.

Sampling station	date time	Latitude Longitude	Fe/colony (nmol)	Protein N/ colony (nmol)	Chl-a/ colony (nmol)	N_2 fix rate (see legend)	N:Fe (mol:mol)	Chl-a:Fe (mol:mol)	mol N fix/ mol Fe/ hour
5	11/6/88 8:30 am	21° 37.258 N 74° 27.390 W	0.140	99	0.069	0.0162	707	0.493	0.12
6	11/6/88 2:08 pm	21° 09.410 N 73° 55.750 W	0.333	204	0.090	0.0124	613	.270	0.04
9	11/7/88 12:43 pm	18° 52.826 N 76° 34.761 W	0.135	75	0.096	0.1384	556	0.709	1.03
16	11/9/88 12:45 pm	16° 50 N 83° 40 W	0.470	78	0.060	0.0914	166	0.128	0.19
25	11/14/88 8:30 am	13° 48.080 N 81° 02.279 W	0.400	99	0.098	0.1248	248	0.245	0.31
26	11/14/88 12:30 pm	13° 27.953 N 81° 13.396 W	0.170	108	0.098	0.1064	635	0.578	0.63
28	11/15/88 8:30 am	10° 22.072 N 82° 42.168 W	0.220	57	0.090	0.6616	259	0.410	3.01
29	11/17/88 10:00 am	10° 36.267 N 82° 26.225 W	0.210	121	0.078	0.0790	576	0.372	0.38
30	11/17/88 1:00 pm	10° 54.576 N 82° 10.330 W	0.175	63	no.data	0.0054	360	no.data	0.03
32	11/18/88 9:30 am	12° 26.203 N 80° 32.711 W	0.220	78	no.data	0.1426	355	no.data	0.65
33	11/18/89 7:00 pm	12° 26.203 N 79° 41.021 W	0.220	117	0.178	no.data	532	0.355	no.data
37	11/20/88 7:00 pm	18° 36.713 N 74° 48.040 W	0.175	117	no.data	no.data	669	no.data	no.data
39	11/21/88 8:30 am	20° 21 N 73° 25.3 W	0.140	51	0.044	0.1064	364	0.314	0.76
mean			0.231	97	0.080	0.136	465	0.387	0.65
Standard Deviation			±0.105	±40	±0.018	±0.181	±180	±0.170	±0.85
n			13	13	10	11	13	10	11

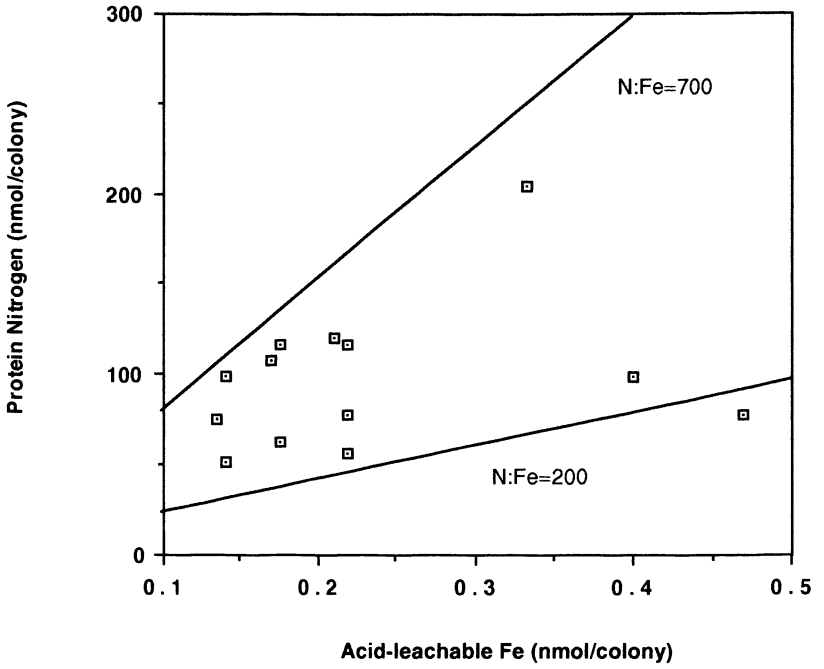


Figure 1. Protein nitrogen compared to acid leachable iron in *Trichodesmium*. Data are the same as in Table 2. The apparent limits of protein nitrogen to iron in these samples were about 700 and 200.

storage in other microorganisms requires nitrogen containing compounds (Stiefel and Watt 1979, Theil 1987) and may be essential to avoid toxic effects of too much iron. Chl-a:Fe ratios vary in a different manner than the N:Fe ratios indicating that *Trichodesmium* can achieve high Chl-a content at low levels of iron. This indicates that either the Chl-a synthesis is less dependent on iron content than cellular accumulation of protein, or that the threshold for the response of Chl-a synthesis to iron is much lower, i.e. more sensitive, than the threshold for general metabolism, including nitrogen fixation.

Although the iron content was not related to the instantaneous nitrogen fixation rate the N:Fe of the cells is in good agreement with calculated values based on the theoretical efficiency of nitrogenase and the iron requirement of the cells for photosynthesis, respiration and nitrogen fixation. The iron requirement for phytoplankton growing at a rate of 1 mol C s^{-1} on N_2 has been calculated to be 79.6 mol Fe (Raven 1988). This theoretical value can be used to calculate the amount of nitrogen the cells could fix per mol of Fe in the cells during a given division time of 5 days. Assuming that the C:N ratio is 6.6

and that the cells only fix nitrogen during 12 hours of every day (in the light), the iron limited cells could fix (1 mol C s^{-1})/(79.6 mol Fe) x (1 mol N/ 6.6 mol C) x 86400 sec/day x 12 h light/24 h day x 5 days to divide = 411 mol N/ mol Fe. This value is extremely close to the measured mean value for the N:Fe= 465 suggesting that it is likely that Fe nutritional content may control the N content of these cells even though the instantaneous measurements of nitrogen fixation rate were seemingly unrelated to the Fe content. A similar approach has been used to argue that all nitrogen transformations in some oceanic regions, not only nitrogen fixation, are potentially under the control of iron content of the autotrophs and herbivores in the community (Rueter, manuscript).

4. Iron Uptake

Iron uptake processes are crucial in the open ocean environment due to the low concentration of soluble iron species and competition for this iron. It has been hypothesized that the exclusive path for iron uptake by phytoplankton, even that iron originating from colloids or other particulates, is through dissolution and subsequent uptake by the cells (Rich and Morel 1990). As a consequence of this dependence on slow dissolution and coordination reactions, phytoplankton in low iron environments would have a large number of iron uptake sites and a high surface area to volume ratio would be advantageous (Hudson and Morel 1990). Based on these predictions, we would expect that iron uptake would be a severe constraint on *Trichodesmium* based on the high requirement and the colonial form which has a very low surface area to total volume. To study this we examined the uptake of soluble iron and the potential for *Trichodesmium* to acquire iron from dust particle directly, a mechanism apparently not available to other phytoplankton.

4.1 UPTAKE OF SOLUBLE IRON

Short term iron uptake studies were performed on natural samples of *Trichodesmium* collected in Caribbean from a variety of depths and at various times of the day. The uptake of iron was saturated by 2 hours, and kinetic studies focused on the first 15 minutes of uptake. The ascorbate rinse was particularly important because these organisms adsorbed iron to the surface very rapidly. The uptake rate could be calculated from data such as shown in Figure 2. These uptake rates in four experiments varied from 0.06 to 0.26 pmol • col^{-1} • min^{-1}. Although the uptake rates and the total iron concentration per colony vary over four collections at different times and depths (Table 3) the maximum iron-specific growth rates only vary within a range of three fold. The estimated doubling times are based only on the iron uptake kinetics and are much shorter than the minimum calculated nitrogen doubling time of 1.5 days (Carpenter et al., 1987) indicating that these cells can take up iron very rapidly compared to their potential growth rates.

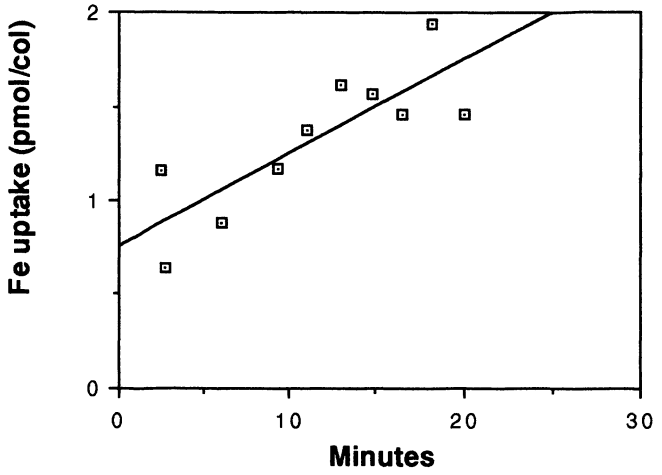


Figure 2. Iron uptake rates calculated from ^{55}Fe uptake. *Trichodesmium* colonies were transferred into 10 mL of filtered seawater in 28 mL capacity Nalgene Oakridge type polycarbonate tubes. Iron as ^{55}Fe in HCl was obtained from New England Nuclear. This stock was diluted with $FeCl_3$ fresh each day to give a working stock with 18 μM total Fe which was then dispensed into incubation tubes. After incubation, cells were rinsed in 0.1 M ascorbate solution for 20 minutes prior to filtering. The filters were dried and counted using either a SAI Technology integrating photometer or a Beckman scintillation counter. Iron uptake was calculated from standards of ^{55}Fe counted in a similar manner. The uptake rate over the entire 20 minute period was 0.05 pmol•col^{-1}•min^{-1}, and the maximum initial uptake over the initial 13 minutes was 0.22 pmol•col^{-1}•min^{-1}.

TABLE 3. Uptake of iron calculated from ^{55}Fe uptake and iron content from ferrozine analysis of colonies at four stations. Iron dependent doubling time is calculated as 0.69 /(Fe specific growth rate).

Time hrs	Depth meters	Fe uptake (pmol col^{-1} min^{-1})	Fe total (pmol col^{-1})	Fe specific growth rate (min^{-1})	time to divide (days)
1300	20m	0.22	68	0.0032	0.15
1320	surface	0.06 to 0.10	71	0.0014	0.34
1600	surface	0.18 to 0.26	59	0.0040	0.12
1000	20m	0.06	22	0.0027	0.18

4.2. INTERACTIONS OF *TRICHODESMIUM* AND DUST

The main source of iron to open ocean systems is the transport of aeolian dust to surface water *Trichodesmium* colonies. Although we have no direct evidence for stimulation of *Trichodesmium* by the iron contained in dust, we have five lines of indirect evidence that support our hypothesis that *Trichodesmium* is able to adsorb and leach iron from dust particles.

4.2.1. *Adsorption of Dust and Movement of the Trichomes.* Dust particles attach extremely readily to *Trichodesmium* colonies. Positively buoyant colonies of *Trichodesmium* could be made to sink by adding dust particles to the container. Microscopic examination of dust particles added to *Trichodesmium* suggested three possible mechanisms that would move dust particles from the exterior surface to the interior of the colony. On a scale that could be observed on many colonies with a dissection scope, the morphology of individual colonies changes over time periods as short as a minute, such as loosening and retightening, making midsection humps, and fraying and retightening of the ends (Figure 3a). At higher magnifications very rapid gliding and rotation can be seen. The relative gliding between two trichomes was measured in the range of $1\mu m \cdot sec^{-1}$. Dust particles were observed to be taken away from the surface of a colony by these gliding and rotational movements. At the end of a fusiform colony, dust attached to the individual trichomes that were exposed, and as these trichomes glided back into the colony the dust is apparently enclosed along with them (Fig 3b). Also at the ends of the colony, rotation of loose ends of trichomes moved one end over a region of another trichome that had dust adsorbed to it (Fig. 3c). On the surface of the midsection of the colony, dust could be incorporated either by lodging in a groove and being covered over or by the rolling action of trichomes due to rotation. Dust was also observed to attach to radial colonies, but no movement was observed that would indicate an incorporation mechanism along the trichomes. These observations of cellular movement suggest that the movement of the trichomes relative to one another in the colonies may help move dust to the interior of the colony where inorganically leached iron could efficiently be taken up, or leaching could be accelerated by organic compounds or the sub-oxic reducing zone (Paerl and Bebout 1988).

4.2.2. *Observations of dust and Trichodesmium by scanning electron microscopy.* *Trichodesmium* colonies were collected and incubated with Saharan dust. Specimens were air dried or fixed with Lugol's solution, rinsed with water to remove salt crystals, sputter coated with Au-Pd and then examined using an ISI-SS40 scanning electron microscope with a Link Analytical AN-10000 Energy Dispersive Spectroscophy unit (Figure 4a). The spectrum for the surface and particles on the surface of the colonies could be analyzed individually. Particles were determined to be either $CaCO_3$ or Al-Si matrix (Figure 4 b,c,d) The Al-Si particles had a high iron content. Some specimens show the ordering of particles into rows in between trichomes, indicating that these particles may be "subducted" into the interior of the colony by trichome rotation. Other specimens show dust particles with a sticky organic coating around them, as if the polysaccharide layers on the cell surface were coating and trapping them. These observations indicate the importance of cell surface activity.

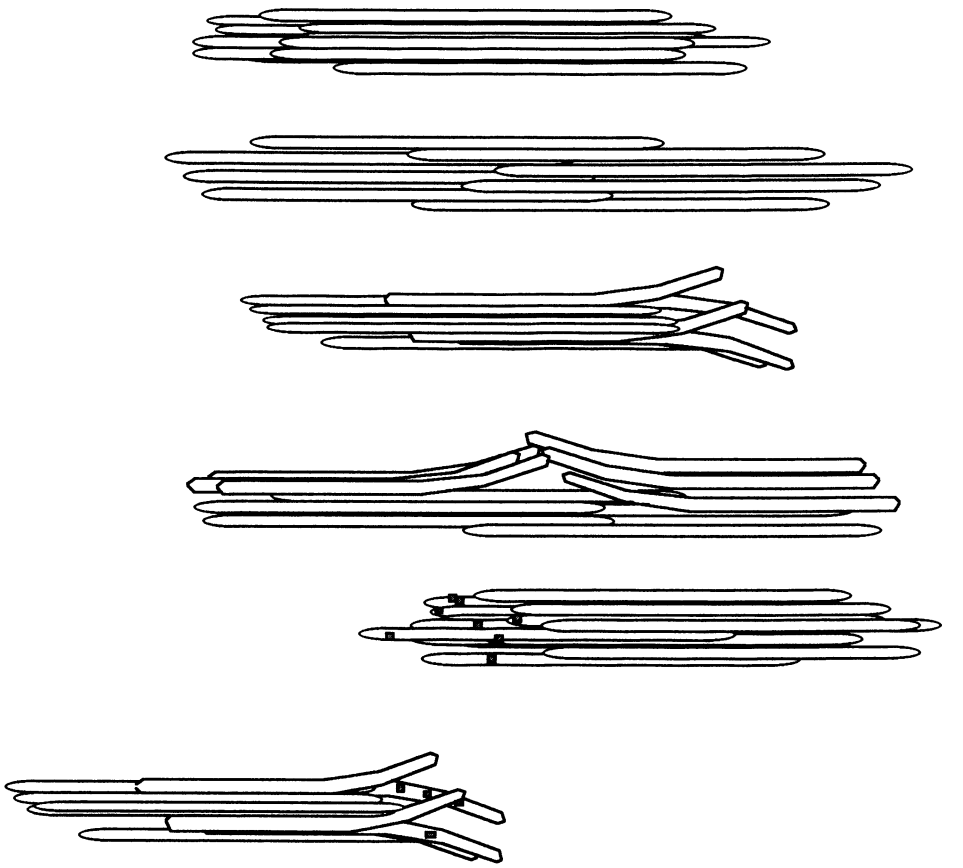
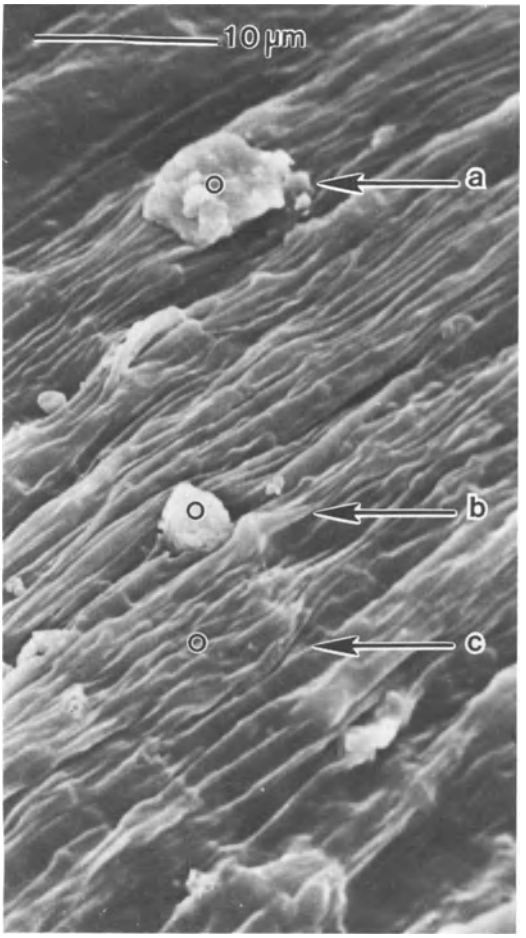


Figure 3. The top four drawings of generalized shape changes that may take place in colonies of *Trichodesmium*. Transitions between these shapes take less than a minute. Drawings based on observations of relative movement of the trichomes relative to one another that may result in the inclusion of dust particles into the interior of a colony, movements include "bulldozer" and end rotation.



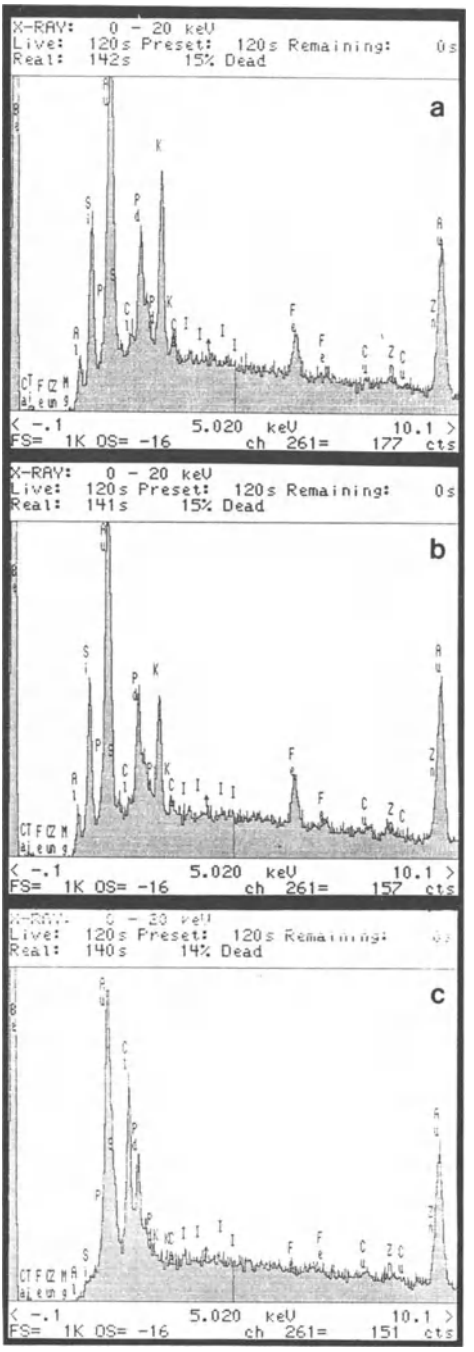


Figure 4. Specimens of *Trichodesmium* were collected in the field and placed on filterpads. Some were air dried and some fixed with Lugol's Iodine. In the laboratory the specimens were washed with distilled water to remove labile salts. Both washed and unwashed samples were examined by SEM with EDS. All *Trichodesmium* specimens showed some accumulation of dust particles, both at the extremities and along the surface. With the SEM, we examined dust particles separately and dust particles on the tissue, and the surface alone without any particulates. The Saharan dust, with its characteristic peaks of iron for K_α and K_β x-ray emission, was easily found at the extremities and between the trichomes along the bundle. The tissues were examined at 20 kV and at 1000-4000 magnification. The EDS unit was operated in the "Mapping" mode for general distribution of elements, and in the "Spot" mode for comparing the individual particles and tissue surface regions.

4.2.3. *Effects of cells on iron chemistry.* *Trichodesmium* was not observed to produce high affinity iron binding compounds, such as siderophores, in response to iron limitation. We used a colorometric assay for iron binding compounds that is sensitive to 5 nM iron binding compound and is unbiased to the type of compound (Schwyn and Neilands 1987). Cultures of *Trichodesmium* NIBB 1067 grown with a range of iron concentrations from none added, to 10^{-7} M, all produced increasing iron binding compounds with time. Even though the growth of these cultures was controlled by iron limitation (Rueter et al. 1990) the increase in iron binding capacity of the medium was more generally related to increase in biomass, increasing more in the iron-replete cultures than in the slower growing iron limited cultures. These observations indicate that these compounds were not iron-repressible and not related to the iron limited status of the cells and thus were not siderophores but probably some by product of growth or cell lysis. Natural samples of *Trichodesmium* that were incubated with and without iron for 2 days also showed no difference in the iron binding capacity of the medium. These measurements, on both cultures and natural samples, indicate that *Trichodesmium* does not seem to make extracellular siderophores.

Concentrated *Trichodesmium* samples often lend a characteristic pink hue to the water. We have extracted this material (called "pink-stuff" by Jones et al. (1986)) from pure samples of *Trichodesmium* that were collected with wide-bore syringes while diving. This indicates that the pink material comes from *Trichodesmium* and not some other components in net tow collections. The pink material can be extracted with either sea-water or distilled water and undoubtedly contains phycobilin pigments but it also has significant iron and dust reactive characteristics. The effect of this pink extract on iron solubility and iron uptake was examined. Addition to ^{55}Fe incubations with *Trichodesmium* resulted in a minor inhibition of the uptake rate to 72% of the control rate. This is an indication that this material does not accelerate transport as a carrier molecule but probably binds to iron in a solely chemical manner. Using two different approaches we demonstrated that it helps to dissolve iron from dust and keep it soluble. The soluble concentration of iron 24 hours after dust addition to filtered seawater was 0.7 μM with the pink material and only 0.2 μM without it, indicating that it can both help solubilize iron from particulate dust and keep it in solution in oxic solutions. The kinetics of the solubilization of iron from dust was examined by following its spectral shift in a solution with added iron and with added dust. Known iron additions caused a spectral shift in the region between 510 and 550 nm. The degree of this shift was used as an indicator of the iron that dissolved from the dust (Fig. 5). Dust was added to the pink extract and a dissolution rate of approximately 0.3 nmol • min^{-1} was observed to be linear for over 300 minutes. The dust exposed to it in this incubation had approximately 50% of the total iron solubilized in 300 minutes.

4.2.4 *Rapid and different responses of Trichodesmium to dust.* The addition of iron to *Trichodesmium*, and the particular form of iron added, has effects that can be seen on the specific proteins. In samples incubated with soluble iron or dust we can see a shift in the relative amounts of Chl-a and phycoerythrin (Table 4). Additions of soluble iron and dust had different effects on the biochemical composition of *Trichodesmium.* In one instance, a 13 hour incubation of a natural sample of *Trichodesmium* with a range of dust and soluble iron resulted in the absolute amount of phycoerythrin increasing more than twice as much with dust additions than with the highest iron. The relative changes in Chl-a was less dramatic (Table 4). These changes resulted in an increase in the PBP to Chl-a ratio with

added dust but not with soluble iron.These patterns for phycobilin to Chl-a were not always evident in *Trichodesmium* colonies that had higher cellular iron concentrations. Thus, given the variability inherent in natural samples of *Trichodesmium*, we are only able to say that these observations on the protein and the pigment composition of *Trichodesmium* indicate that these organisms can respond, in time periods less than or equal to their division times, in different ways to soluble iron or dust additions.

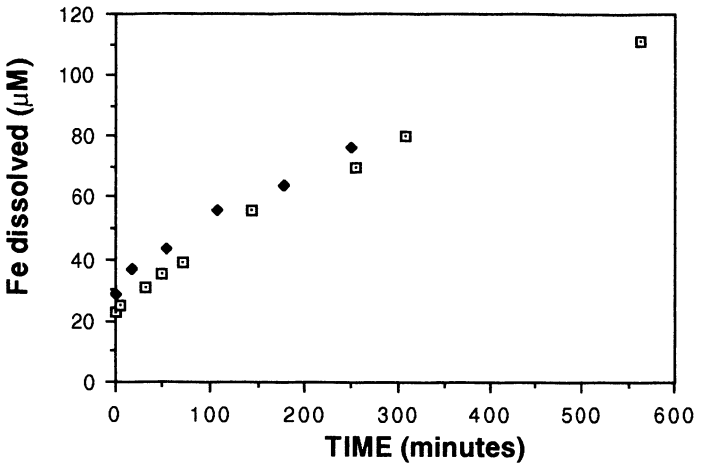


Figure 5. Calculated dissolved iron concentration in a concentrated solution of the pink extract and dust. The absorbtion shift of the "pink-stuff" was measured for known concentrations of iron added. (The linear regression from this known addition was $\Delta abs = -0.006 + 4.5x10^{-4}$ (Fe) R=0.99.) The same solution was used as a reference.

TABLE 4. Short term response (13 hours) of Chl-a and phycoerythrin (PE) synthesis in *Trichodesmium* to added iron. Phycoerythrin was estimated by fluorescence after treating the cells with 50% glycerol.

treatment	Chl-a	PE
+ 20 nM Fe	.065	1.4
+100 nM Fe	.073	1.4
+ 500 nM Fe	.080	2.0
+ 1 mg dust	----	2.2
+ 5 mg dust	.058	3.0
+25 mg dust	.067	4.4

4.2.5 *Probability of dust colliding with colonies.* If dust adsorption is an important process for *Trichodesmium* it must be feasible that enough dust can be intercepted in this manner to make a difference to the iron nutrition of this organism. The minimum probability that a dust particle will hit a *Trichodesmium* colony in the open ocean water column can be estimated based on several assumptions. Assume that the probability of a dust particle colliding with a *Trichodesmium* colony over a very short fall (such as 10 cm) is simply proportional to the relative cross-sectional area occupied by *Trichodesmium* colonies. This means that given a population density of one colony per liter and colonies with a radius of only 1 mm that the probability of a dust particle not hitting a colony in a 10 cm fall (because a liter is 1000 cm^3) is 99.96%. However the cumulative probability of a particle not hitting a colony after falling through 2000 of these 10 cm sections in a 200 m water column is only 44.9% (which equals 0.9996^{2000}). This means that there is a better than 50% chance that every dust particle will be intercepted by a *Trichodesmium* even for these conservative assumptions for the density and size of *Trichodesmium.* From this calculation it is probable that the majority of dust particles will hit *Trichodesmium* and from our observations if dust hits, it sticks. Smaller dust particles remain suspended in the water column with a residence times of on the order of 20 days greatly increasing the probability that they will collide with a *Trichodesmium* colony.

In summary, we have five lines of evidence that indicate dust may be an important form of iron for *Trichodesmium* nutritional demands: 1) dust adsorbs to *Trichodesmium* readily and there are movements of the filaments that may internalize or trap these particles; 2) iron containing particles can be identified on the surface of *Trichodesmium*, using EDS - scanning electron microscopy and some of these particles are wedged between trichomes or covered with an organic coating at the cell surfaces; 3) although *Trichodesmium* was not observed to make siderophores, colonies are capable of producing some compounds that accelerate the dissolution of iron from dust and keep that iron soluble; 4) *Trichodesmium* colonies respond rapidly to additions of dust and the response is slightly different, in terms of the Chl-a to PBP ratio, than when given soluble iron; 5) in the open ocean, it is more probable for a dust particle to hit a *Trichodesmium* colony than it is for that particle to sink through a water column indicating that adsorption of particles is at least feasible.

5. Geochemical Significance of *Trichodesmium*

Biogeochemical cycles are controlled by organisms, sometimes specific organisms. In the tropical oligotrophic oceans, it is already recognized that *Trichodesmium* makes a significant contribution to the nitrogen nutrition of the entire community (Carpenter 1983, Knap et al. 1986). I have argued here that *Trichodesmium* may also play a crucial role, i.e. that it is a COG, in the introduction of iron into the system through its ability to intercept, adhere to and leach dust particles. The dual roles of *Trichodesmium* in both the geochemistry of nitrogen and iron can illustrated with the North Tropical Atlantic as an example. The total primary productivity in the North Tropical Atlantic (NTA) has been estimated to be from 6 mol C $m^{-2}y^{-1}$ (Menzel and Ryther 1960) to 7.5 (Knap et al. 1986). This translates to a total nitrogen productivity in the range of 0.8 to 1.1 mol N m^{-2} y^{-1} [using either the Redfield ratio (Redfield 1958) or newer estimates of 122:16 (Takahashi et al. 1985)]. Of this approximately 1 mol N $m^{-2}y^{-1}$ a range of from 0.08 to 0.35 mol N

m^{-2} y^{-1} [Knap et al 1986, Platt and Harrison 1985) has been called "new production" based on NO_3^- flux into the surface waters and nitrate dependent growth. Nitrogen fixation in the NTA may also make a small contribution to the new production of nitrogen. Estimates for nitrogen fixation from 0.01 to 0.05 mol N $m^{-2}y^{-1}$ (Carpenter 1983) indicate that nitrogen fixation may contribute 1-5% to the total nitrogen budget. The amount of iron necessary to support this amount of nitrogen fixation can be calculated from the N:Fe = 465 for *Trichodesmium* (Table 2). Using the higher estimate of 0.05 mol N m^{-2} y^{-1} for nitrogen fixation divided by 465 gives 1 x 10^{-4} mol Fe m^{-2} y^{-1} of iron nutrition necessary. The total iron input to the North Tropical Atlantic is 0.002 mol Fe m^{-2} y^{-1} (Jickells et al. 1984). If we accept that only 10% or less of this is dissolves and becomes biologically available (Hodge et al. 1978, Moore et al. 1984), this would lead to 2 x 10^{-4} mol m^{-2} y^{-1} of biologically available iron, in which case half of this must go to *Trichodesmium* to support nitrogen fixation. On the other hand, if we consider the possibility that *Trichodesmium* can acquire iron directly through interaction with dust particles, then *Trichodesmium* would be contributing new iron as well as new nitrogen to the system. This new iron may be very important in determining some aspects of community structure or supporting iron-dependent processes in the underlying deep water biota (Rueter manuscript). In either case, *Trichodesmium* would have a major effect on the iron availability in the system and a substantial long term effect on the total nitrogen in the community.

6. Conclusions

Iron-dependent metabolic processes are crucial for the growth of *Trichodesmium.* In particular, the large amount of iron required just to support nitrogen fixation makes *Trichodesmium* and other diazotrophs very dependent on iron nutritional availability. Trichodesmium can take up soluble iron very rapidly but may be at a competitive disadvantage for iron uptake compared to other phytoplankton due to the very low surface to volume ratio of the colonies. Several characteristics of the interaction between *Trichodesmium* and aeolian dust led us to hypothesize that this organism may be able to intercept, adsorb, and solublize the iron from dust and thus allow a unique path for iron acquisition. *Trichodesmium* provides important new nitrogen to oligotrophic oceans and, possibly just as important, its growth may account for approximately one-half of the flux of biologically available iron. Many characteristics of *Trichodesmium* including colony formation and buoyancy should be considered in the importance of iron nutrition to this organism.

7. Acknowledgements

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TRICHODESMIUM: ESTABLISHMENT OF CULTURE AND CHARACTERISTICS OF N_2-FIXATION

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ABSTRACT. Details of techniques for isolation and culture of *Trichodesmium* from Kuroshio waters, including special care for maintaining a stable culture, are explained. Properties of this isolate grown under artificial conditions indicate that the organism in culture is similar to *Trichodesmium* in the natural habitat. Some characteristic features of N_2-fixation in this organism are also reviewed.

1. Introduction

Establishment of stable cultures of *Trichodesmium* has been attempted by many marine biologists. Ramamurthy (1972) reported the culture of *Trichodesmium erythraeum* using a sea water medium enriched with many kinds of nutrients including vitamins, soil extracts and plant hormones. This first success, however, has never been confirmed by other investigators. Later, Carpenter and McCarthy (1975) succeeded in keeping *Trichodesmium thiebautii* alive in the enriched sea water medium of Guillard and Ryther (f-medium, 1962), but they failed to obtain active growth. As reported previously, we successfully isolated and cultured *Trichodesmium sp.* from Kuroshio waters (Ohki and Fujita, 1982) in the sea water medium of Guillard and Ryther. Soon after, a stable culture in the complete synthetic medium was also obtained (Ohki et al., 1986, 1991a). Recently, another research group has also succeeded in the isolation of *Trichodesmium sp.* from the open sea near Hawaii, and has kept it under artificial conditions for more than a half year, but without rapid growth (R. Letelier, personal communication). In this chapter, we describe our strategy for stabilizing the culture of *Trichodesmium* in artificial medium, and review some physiological and biochemical characteristics of the strain *Trichodesmium sp.* NIBB1067 isolated from Kuroshio waters and grown under these conditions. Problems arising from comparison of organisms grown in the laboratory culture with those in the natural habitat are also discussed.

2. Culture of *Trichodesmium*

2.1. ISOLATION FROM THE NATURAL HABITAT

2.1.1.*Sample for Isolation. Trichodesmium* is found as a large spherical or bundle-shaped colony

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in the natural habitat. Since colonies contain many other micro-organisms (cf. Paerl et al., 1989) and cells in the colony are generally aged and nutrient starved, they cannot be used for the source of isolation. Small bundle-shaped colonies or separate trichomes are much more physiologically active (Ohki and Fujita, 1988), so that the best source for isolation are separate trichomes. For this reason, collection of separate trichomes from water samples is desirable. Trichomes can be collected with gentle filtration through nylon mesh. When cells are to be collected by a towing net, samples must be diluted immediately after each tow. Trichomes or colonies are then quickly separated by pipette into filtered sea water.

Most *Trichodesmium* colonies found in Kuroshio waters in the summer have larvae of *Microsetella gracilis* attached (cf. Ohki and Fujita, 1982), which graze on *Trichodesmium* (Ohki, Ishimaru, Egawa, Wada and Fujita, unpublished data). Another grazer, the copepod *Macrosetella gracilis* (Roman, 1978), was found in *Trichodesmium* colonies obtained from the Caribbean Sea (U.M. O'Neil, personal communication). It is necessary to examine each colony carefully under the microscope, and remove these organisms before attempting isolation.

2.1.2.*Medium*. Enriched sea water (f-medium, Guillard and Ryther, 1962) is suitable for the primary culture for the isolation. The organism can survive for long time periods even when it cannot grow. However, the concentration of nutrients should be reduced to 1/40 of the original. Survival cannot be expected at high nutrient concentrations. To prevent growth of contaminating phytoplankton such as *Synechococcus* and diatoms, combined nitrogen ($NaNO_3$) and Na_2SiO_3 should be removed from the medium. Rueter et al. (1979) have observed that *Trichodesmium* in the natural habitat is very sensitive to copper. Other heavy metals are probably toxic also. Analytical grade chemicals must be used. Even analytical grade, phosphate reagents often contain toxic metals. In this case, metal ions should be removed with a Chelex-100 column (cf. Morel et al., 1979). All glassware should be soaked in hydrochloric acid to remove contaminating heavy metals.

2.1.3.*Other conditions*. One to three small colonies, each of which consists of 10 to 30 trichomes, were used to inoculate a test tube containing 15 to 20 ml of the medium. Both incandescent light and fluorescent light (10 to 15 W/cm^2, 8 to 12 hr light/ 14 to 12 hr dark cycle) can be used for the light source. A moderate light intensity (10 to 15 W/cm^2) is desirable to obtain a stable culture. However, the best results were obtained when the cells were grown under weak natural light conditions. Temperature was kept at about 25°C.

It is important that cells are transferred to fresh medium every 2 to 3 weeks, even when they have not started to grow. *Trichodesmium* cells remain alive without growth for very long periods especially when they are transferred for the first time from the natural environment to culture (Ohki and Fujita, 1982). We observed cells which survived for more than 7 months. After starting active growth, transfer to the new medium must be made much more frequently so as to keep low cell density.

2.1.4.*Isolation of Trichodesmium sp. NIBB1067 from Kuroshio Waters*. The above techniques have allowed us to successfully isolate and culture *Trichodesmium* sp. from Kuroshio waters. Trichomes collected with a towing net were first inoculated into f-medium of Guillard and Ryther (1962) with reduced nutrients (1/40). Trichomes were transferred to fresh medium every 2 to 3 weeks until cells started to grow. Active growth in test tubes occurred after about 40 days. Once cells were actively growing, they were not necessarily sensitive to a high nutrient concentration, and we obtained rapid growth in nutrient concentrations as high as f/10. In the active growing culture, bundle-shaped colonies split into single trichome upon transfer into fresh medium. When these separate trichomes were inoculated into fresh medium, the number of tricomes increased

without colony formation. Colony formation occurred only at the late linear to stationary growth phases, when trichome density became high (Ohki and Fujita, 1982).

With use of the separate trichomes in the exponential growth phase in f/10 medium, we tested the effect of each component of artificial sea water on the growth of *Trichodesmium* (Ohki et al., 1986). The organic pH buffers commonly used for the artificial medium such as tris-aminomethane and glycylglycine were very toxic, and killed *Trichodesmium* within several days. The pH of the medium must be buffered by $NaHCO_3$-NaOH or -HCl combination for the stable growth of *Trichodesmium*. As described in 2.1.2, Na or K salt of inorganic phosphate strongly inhibited *Trichodesmium* growth. This toxicity of the phosphate was reduced by purification with a Chelex-100 column (Ohki et al., 1986). However, phosphate higher than 10^{-4}M still inhibited cell growth somewhat. Sensitivity to Cu^{2+} was reported by Rueter et al. (1979). Iron higher than 5×10^{-7}M also showed an inhibitory effect on cell growth (cf. Rueter et al., 1990). These features suggest that *Trichodesmium* is very sensitive to heavy metals. The culture medium should be prepared with special care to remove metals as much as possible. This is different from the requirements for culture of coastal phytoplankton. Since very high sensitivity to heavy metals is also found in other phytoplankton from the open sea, it is probably a common character of the organisms living only in these waters.

The Ca^{2+} concentration was found to be very important in successful culture of *Trichodesmium*. Cell-lysis occurred rapidly when the Ca^{2+} concentration in the medium was lower than 4.6×10^{-3}M. However, *Trichodesmium* became tolerant of low salinity (20‰) when Ca^{2+} concentration was high enough (9.2×10^{-3}M, Ohki et al., 1986). The requirement for a high concentration of Ca^{2+} was not replaced with an increase in Mg^{2+} concentration, indicating that the Ca^{2+} requirement is independent of Mg^{2+} concentration. This is in contrast to the Ca^{2+} requirement of coastal species (cf. Provasoli et al., 1957). Combined nitrogen also supported active growth; however, ammonia at higher than 2×10^{-5}M or urea at higher than 5×10^{-4}M was toxic. The latter may cause an accumulation of ammonia within the cell.

Following the procedures described above, we succeeded in maintaining a stable culture of *Trichodesmium* in Aquil medium (Morel et al., 1979) under a slight modification. Filtration or autoclaving can be used for sterilization of the medium. In the latter case, $CaCl_2$ and $SrCl_2$ must be autoclaved separately and added after cooling to avoid precipitation. The composition of the modified Aquil medium is shown in Table 1. We have not investigated the effect of vitamins and some of microelements, which are present in the original Aquil medium.

2.2. PROPERTIES OF *Trichodesmium* sp. NIBB1067 GROWN UNDER ARTIFICIAL CONDITIONS

Trichodesmium sp. NIBB1067 in modified Aquil medium can use different nitrogen sources (Fig. 1). Values of δ15N of cellular-N have revealed that *Trichodesmium* sp. NIBB1067 utilizes urea and/or nitrate as a nitrogen source when these combined nitrogen sources are present in the medium (Ohki, Egawa, Wada and Fujita, unpublished data). The cell growth was not affected by nitrogen source. The maximum growth constant in the exponential growth phase was about 0.18/day under illumination with a fluorescent lamp (15 W/m^2, 12hr light/ 12 hr dark).

As shown in Fig. 2, morphological characteristics of *Trichodesmium* sp. NIBB1067 observed under light (Fig. 2A) and electron microscopes (Fig. 2B and C) are the same as those observed with cells of the natural population (cf. Van Baalen and Brown, 1960, Gatt et al., 1984). The average diameter of trichomes ($9.2 \pm 0.63\mu$m) was similar to that observed with *Trichodesmium thiebautii* in the natural habitat (cf. Ohki and Fujita 1982). However, the amount of gas vesicles seemed to be slightly smaller in the cultured cells than in the cells grown in the natural habitat.

Table 1. Composition of modified Aquil medium used for the culture of *Trichodesmium*.

Component	Concentration (M)
NaCl	4.2×10^{-1}
$MgCl_2 \cdot 6H2O$	5.5×10^{-2}
K_2SO_4	2.2×10^{-2}
KCl	9.4×10^{-3}
$CaCla_2$	9.2×10^{-3}
$NaHCO_3$	2.4×10^{-3}
KBr	8.4×10^{-4}
H_3BO_3	4.9×10^{-4}
KH_2PO_4	1.0×10^{-4}
NaF	7.1×10^{-5}
$SrCl_2 \cdot 6H_2O$	6.4×10^{-5}
Fe-EDTA	1.0×10^{-7}
$MnCl_2 \cdot 4H_2O$	2.3×10^{-8}
$ZnSO_4 \cdot 7H_2O$	4.0×10^{-9}
$CoCl_2 \cdot 6H_2O$	2.5×10^{-9}
$(NH_4)_3Mo_7O_{24} \cdot 4H_2O$	1.5×10^{-9}
$CuSO_4 \cdot 5H_2O$	1.0×10^{-9}
Thiamine-HCl	6.0×10^{-7}
Biotin	4.0×10^{-9}
B_{12}	7.4×10^{-10}

Medium pH is adjusted to 8.0 with NaOH. $NaNO_3$ 2×10^{-3}M is added, if necessary.

Trichodesmium has been reported to contain a unique phycoerythrin, which shows three absorption peaks (500, 547 and 565 nm) in the visible wavelength region (Fujita and Shimura, 1974). The absorption of intact cells and the crude phycoerythrin fraction of *Trichodesmium* sp. NIBB1067 (Fig.3) have indicated that spectroscopic characteristics of the phycoerythrin in this organism are the same as those observed with *Trichodesmium* in the natural habitat (Fujita and Shimura, 1974). The chromophore composition of this phycoerythrin estimated by the absorption spectrum following denaturalization in acidic urea (8.0 M, cf. Glazer and Hixon, 1977; Ong et al., 1984), indicates that the molar radio of phycoerythrobilin to phycourobilin is 3. Table 2 shows the pigment composition in cells at the exponential growth phase. Pigment composition, abundance on a per cell basis and stoichiometry among pigments, was not affected by the nitrogen source for cell growth.

Table 3 shows the maximum rates of photosynthesis and light-dependent acetylene reduction of this organism. N_2-fixation activity (acetylene reduction) was found only in cells grown in medium without combined nitrogen. The maximum activity for N_2-fixation was observed with cells at the exponential growth phase (Ohki and Fujita, 1986).

Zehr and McReynolds (1989) sequenced a 395 bp fragment of the *nifH* gene amplified from the DNA of *Trichodesmium thiebautii* collected from the Caribbean Sea. The sequence from the DNA of *Trichodesmium* sp. NIBB1067 was found to be 98% identical at both the protein and nucleotide levels to that of *Trichodesmium thiebautii* from the Caribbean Sea (Zehr et al.,1990; Zehr, in this issue). This homology is very high when compared with that between DNAs of

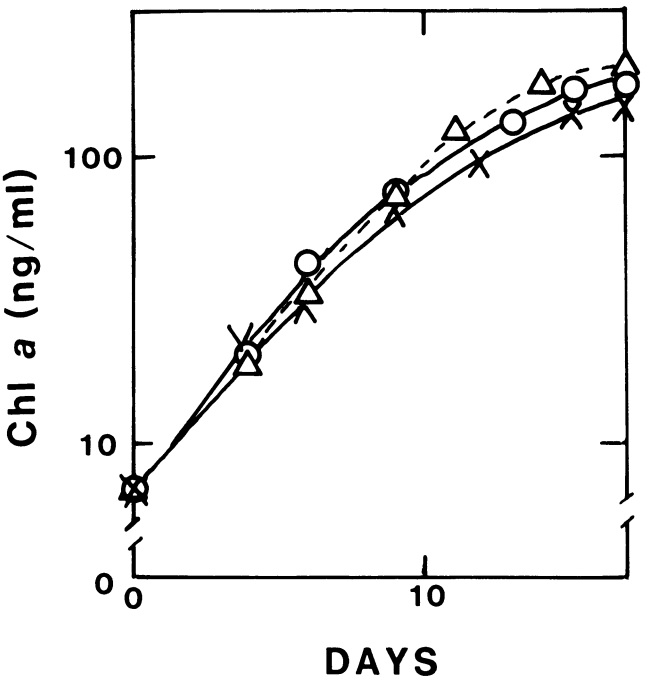

Figure 1. Growth of *Trichodesmium* sp. NIBB1067 with different nitrogen sources. Cells were grown in modified Aquil medium without combined nitrogen (open circles), with 2 x 10^{-3}M $NaNO_3$ (crosses), and with 5 x 10^{-4}M urea (triangles) under illumination with fluorescent light (day-light type, 15 W/m^2,12 hr light/ 12hr dark cycle) at 26°C.

Trichodesmium thiebautii and of the heterocystous cyanophyte, *Anabaena* sp. PCC7120 (79% and 84% for the nucleotide and protein levels, respectively, Zehr and McReynolds, 1989). This degree of homology indicates that *Trichodesmium* sp. NIBB1067 is genetically very similar to *Trichodesmium thiebautii* from the Caribbean Sea.

3. Study of Regulation of Nitrogenase Activity with Cultured Cells

Since cells grown under artificial conditions show a wide variation in activity for N_2-fixation depending on the nitrogen source in the medium, we have analyzed the regulation of nitrogenase activity with cells grown under various conditions. The following is a brief explanation of results obtained from such trials.

The abundance of Fe- and the MoFe-proteins of nitrogenase was determined by immunoblotting to examine the relationship between the activity for N_2-fixation and the abundance of nitrogenase (using heterologous antisera raised against the Fe- and the MoFe-proteins of *Rhodospirillum rubrum*, generously provided by Dr. P.W. Ludden at University of Wisconsin). The antisera showed a very high reactivity to the nitrogenase of *Trichodesmium* sp. NIBB1067 (cf. Ohki et al.,

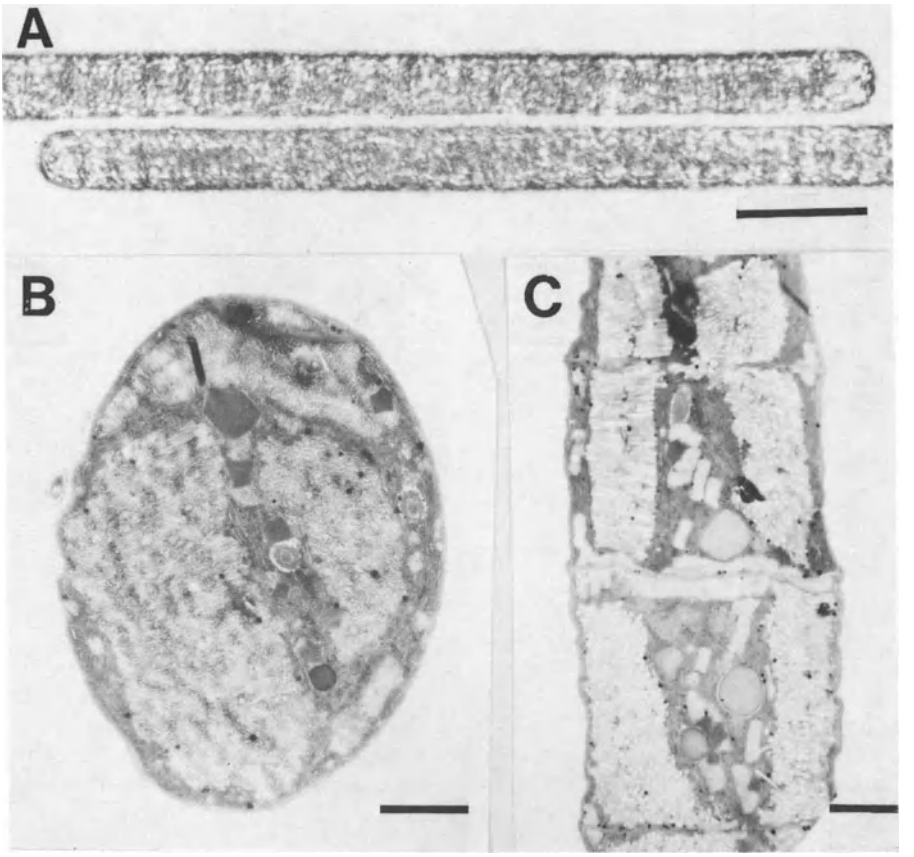

Figure 2. Micro-photographs of Trichodesmium sp. NIBB1067. Cells grown under N_2-fixation conditions used for examination under light microscope (A) and under electron microscope (B and C). For the electron-microscopic observation, cells fixed with 1% glutalaldehyde and 1% OsO4 were embedded in Epon 812 resin. Sections were stained by uranyl acetate. B, cross section of the cell; C, longitudinal section of the trichome. Bars indicated 20 μm for A and 1 μm for B and C.

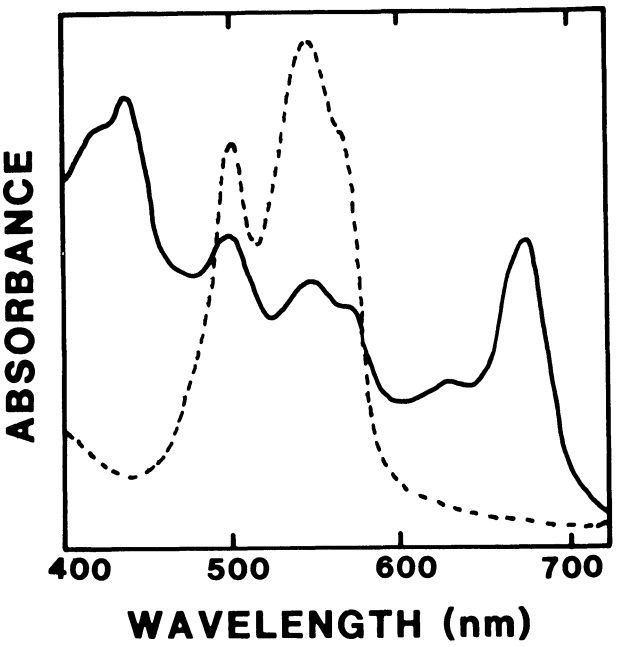

Figure 3. Absorption spectra of intact cells (solid line) and crude phycoerythrin fraction (dashed line) of *Trichodesmium* sp. NIBB1067 grown under N_2-fixation conditions. Cells were placed on polycarbonate filter (Nucleopore, No.111123). Phycoerythrin fraction was obtained by a hydroxyapatite column chromatography, and dissolved in 0.01M phosphate buffer (pH 6.2).

1991b). Nitrogenase activity was observed only when *Trichodesmium* sp. NIBB1067 was grown in the medium without combined nitrogen. No activity was detected in cells grown with nitrate, ammonia or urea (Ohki et al., 1991b). As shown in Fig. 4, neither the Fe- nor the MoFe-proteins were detected in cells grown with urea as the nitrogen source. In contrast, cells grown with nitrate or ammonia contained the Fe- and the MoFe-proteins, indicating that the enzyme is synthesized but it is inactive in these cells. Patterns of SDS-PAGE for the MoFe-protein were the same from cells which were active or inactive in N_2-fixation (Fig. 4A, Ohki et al., 1991b). However, the Fe-protein in cells grown with nitrate or ammonia migrated on a SDS-PAGE differently from the protein in the active cells. In this case, it formed a single protein band with apparent molecular mass of about 40 kDa, while the Fe-protein in active cells showed an additional band which migrated with apparent molecular mass of about 38 kDa (Fig. 4B, Ohki et al., 1991b). Inactivation of N_2-fixation has been observed when active cells were incubated for a short time (30 min) in the dark or in the light with high concentration of O_2 or urea (data, not shown). In such cases, inactivation was found to be always accompanied with disappearance of the band of low molecular mass in SDS-PAGE of the Fe-protein. These features indicate that the nitrogenase activity of *Trichodesmium* is also regulated through the modification of the Fe-protein. The form of the "lower molecular mass" must be active, and "higher molecular mass", inactive enzyme. Regulation of nitrogenase activity by post-translational modification of the Fe-protein has been reported for

Table 2. Pigment composition of *Tricodesmium* sp. NIBB1067 grown with N_2 or Nitratre as the nitrogen source.

N-source	Phycobiliprotein			Chlorophyll *a* (10^{-16} mol/cell)
	PE[1]	PC[2] (10^{-18} monomer mol/cell)	APC[3]	
N_2	11.85 ± 1.65	4.36 ± 0.61	5.67 ± 0.79	1.28 ± 0.18
$NaNo_3$	10.26 ± 1.03	3.63 ± 0.37	4.17 ± 0.42	1.13 ± 0.11

Abundance of pycobiliproteins was measured spectrophotometrically after solubilization of 0.05 M phosphate buffer (pH 6.2). Absorption coefficients of Hattori and Fujita (1959) were used for determination of phycocyanin and allophycocyanin. Phycoerythrin concentration was calculated from the chromophore concentration measured in acidic urea (8M, Glazer and Hizon, 1977, Ong et al., 1984).

[1] phycoerythrin
[2] phycocyanin
[3] allophycocyanin

Table 3. Maximum activities for photosynthesis and N_2- fixation (acetylene reduction) observed with *Trichodesmium* sp. NIBB1067 at the exponential growth phase.

Activity	mol/mol Chl *a* ·h	10^{-20} mol/cell·h
Photosynthesis[1]	253	21.50
Acetylene reduction[2]	46.2	3.93

[1] Oxygen evolution was measured with a Clark-type oxygen electrode.
[2] Light-dependent activity of acetylene reduction.

some of the photosynthetic bacteria (cf. Zumft, 1985). In such cases, nitrogenase activity is regulated by the reversible modification of the Fe-protein; the apparent molecular mass of inactive Fe-protein becomes 1.5 to 5.0 kDa higher than that of active enzyme on SDS-PAGE. Modification of the Fe-protein in *Rhodospirillum rubrum* has been known to be due to an ADP-ribosylation (Pope et al., 1985). Simultaneous changes in the apparent molecular mass of the Fe-protein and nitrogenase activity have also been reported for cyanobacteria, though occurrence of ADP-ribosylation has not been demostrated (Ernst et al., 1990; Reich and Böger, 1989; Smith et al., 1987). In conclusion, the nitrogenase activity in *Trichodesmium* sp. NIBB1067 is regulated by the growth environment in two ways: modification of the Fe-protein as a "short-term" regulation, and regulation of protein synthesis at or before translational level as a "long-term" regulation.

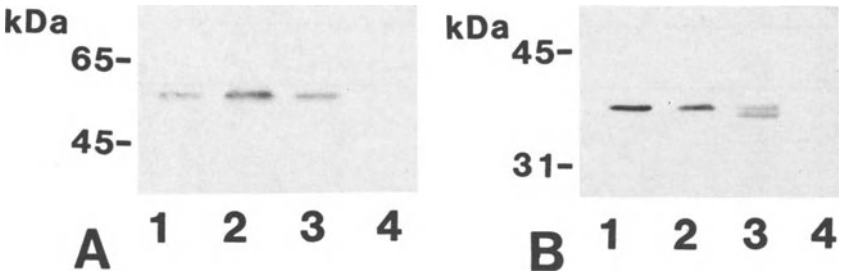

Figure 4. Western immunoblot analysis of cell extracts obtained from *Trichodesmium* sp. NIBB1067 grown with various nitrogen sources. Antisera raised against the MoFe-protein (A) or the Fe-protein (B) of nitrogenase of *Rhodospirillum rubrum* were used as probes. Cells were grown with N_2 (lane 1), with 2 x 10^{-3}M of $NaNO_3$ (lane 2), with 2 x 10^{-5}M of NH_4Cl (lane 3) and with 5 x 10^{-4}M of urea (lane 4).

4. *Trichodesmium* in Artificial Culture and in the Natural Habitat

Although the underlying mechanism of regulation of nitrogenase may be the same in culture as in natural populations of *Trichodesmium*, the expression of nitrogenase and nitrogen fixation can differ. The following is one example. Diurnal changes in nitrogenase activity of *Trichodesmium* was observed in the natural habitat (Saino and Hattori, 1978; Capone et al., 1990). The activity for N_2-fixation in the light is typically insignificant before sunrise, and appears at 1 to 2 hours after sunrise. The activity becomes maximum around noon, then it gradually decreased and disappears at the time of sunset. Capone et al. (1990) reported that for the natural population in the Caribbean Sea, the abundance of the Fe-protein in cells is insignificant 2 to 4 hours before sunrise, but rapidly increased after sunrise. These results suggest that diurnal change of the activity is attributed to dark-degradation and the light-dependent *de novo* synthesis of nitrogenase. A similar variation of activity under a light/dark cycle can be observed with cells of the cultured strain, *Trichodesmium* sp. NIBB1067 (Ohki, Zehr and Fujita, unpublished data). However, both the Fe- and the MoFe-proteins of nitrogenase are present even after dark incubation for 18 hours. The Fe-protein remains in the "high molecular mass" form, which must be inactive, in the dark. The "low molecular mass" form, which corresponds to the active proetin, appears after illumination for 1 to 3 hours (data, not shown). Thus, dark-inactivation and light-activation occurs only at the post-translational modification of the Fe-protein in the artificial culture system. The sea area where *Trichodesmium* forms a dense population is oligotrophic. Nutrient limitation may be more severe in the natural habitat than in artificial culture. This difference may change characteristics of turnover of the enzyme, synthesis and/or degradation. Synthesis of the enzyme may be reduced due to a more strict limitation of nutrient supply. As a result, degradation is more clearly observed than in cells grown in artificial culture. On the other hand, the synthesis of the enzyme in cells grown in artificial culture may be active enough to maintain a high abundance of the Fe-protein, but in an inactive form in the dark. It is likely that the level of the active Fe-protein is determined in two ways: 1) the turnover of the protein due to synthesis and degradation, and 2) turnover between active and inactive forms due to post-translational modification (Table 4).

Table 4. Nitrogenase activity of *Trichodesmium* grown in the artificial culture and in the natural habitat.

Origen	Organism	Activity 10^{-15} mol N_2/cell·hr
Laboratory Culture	*Trichodesmium* sp. NIBB1067	43 - 135[1]
Central North Atlantic[2]	*Trichodesmium thiebautii*	4 - 29
Sargasso Sea[3]	*Trichodesmium thiebautii*	3 - 36

[1] Values obtained under assumption of a 3:1 conversion for ethylene to N (cf. Saino, 1978)
[2] Carpenter and McCarthy (1975).
[3] McCarthy and Carpenter (1979).

Though available data are rather limited, the activity in the cultured cells is almost 10 times higher than those in cells in the natural habitat. Lower nitrogenase activity in cells in the natural habitat may be due to the physiological state of the cells, which may be similar to that of cells at the late linear to the stationary growth phase in artificial culture. Alternatively, nutrient limitation in the natural habitat may cause a low abundance and low activity of nitrogenase even in actively growing cells. Development of suitable indices to indicate such different physiological states of *Trichodesmium* is necessary.

Development of the culture of *Trichodesmium* in artificial medium has made it possible to perform physiological, biochemical and genetic studies using cells grown under defined conditions. However, the present status of the culture system is not sufficient for an adequate understanding of phenomena observed in the natural habitat. Further development of the culture system is necessary so as to be able to simulate the growth conditions in the natural habitat. *Trichodesmium* living in the natural habitat is often associated with other microorganisms (Pearl et al., 1989). The relationship between *Trichodesmium* and those organisms must be important to understand the role of *Trichodesmium* in the marine ecosystem. Isolation and characterization of these micro-organisms will also be important for understanding the ecology and physiology of *Trichodesmium* in the environment.

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THE TOXICITY OF *TRICHODESMIUM* BLOOMS FROM CARIBBEAN WATERS

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ABSTRACT. Bloom samples of the marine pelagic cyanobacteria *Trichodesmium thiebautii* and *Trichodesmium erythraeum* were collected off St. Thomas in the US Virgin Islands in April 1990 and at locations throughout Caribbean waters during January and February 1991. Samples containing *T. thiebautii* were highly neurotoxic to mice with LD_{50} values (intraperitoneal) ranging from 5 to 85 mg bloom dry weight per kg body weight. Toxicity was characterized by severe convulsions and death within 2-30 minutes by respiratory arrest. HPLC diode array analysis of extracts of the toxic bloom samples did not reveal UV absorption spectra corresponding to known cyanobacterial toxins. Further characterization of *Trichodesmium* bloom toxicity was achieved using invertebrate assays, bacterial luminescence and enzyme assays and HPLC analysis. The possible biological significance of the *Trichodesmium* bloom toxic factor(s) is discussed.

1. Introduction

Bloom-forming cyanobacteria (blue-green algae) in fresh- and brackish waters are widely recognised as being capable of producing toxins. A substantial literature exists on the molecular properties of the toxins, which include peptides, alkaloids, organophosphorus compounds and lipopolysaccharides, and on the deaths of animals attributed to these toxins in many countries (Carmichael, 1988; Gorham and Carmichael, 1988; Codd et al., 1989a,b). Cyanobacterial toxins are also hazards to human health (Falconer, 1989, 1991; Codd et al., 1989a,b; Turner et al., 1990). The genera most often implicated in poisoning incidents and encountered in the toxicity testing of blooms are *Microcystis*, *Anabaena*, *Oscillatoria* and *Aphanizomenon*. These are usually regarded as freshwater cyanobacteria, although they osmoadapt to sodium chloride and can grow in brackish waters.

The first documented case of animal poisonings and human illness due to contact with/ingestion of cyanobacterial blooms in the scientific literature involved the brackish water planktonic *Nodularia spumigena* in Lake Alexandrina at the mouth of the Murray River,

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Australia (Francis, 1878). Since then, animal poisonings attributed to toxic *N. spumigena* blooms have been reported along the Swedish, Finnish, German and Danish coasts of the Baltic Sea (see Sivonen et al., 1989). Coastal/estuarine habitats in Australia, New Zealand and the United Kingdom have also been found to support toxic blooms of *N. spumigena* (e.g. Runnegar et al., 1988; K.A. Beattie and G.A. Codd, in preparation).

Marine coastal habitats around islands in the Pacific have yielded a range of toxic cyanobacteria which have been associated with contact dermatitis or swimmers' itch (Moore, 1984). These include *Lyngbya majuscula*, *Schizothrix calcicola* and *Oscillatoria nigroviridis* which are mainly found as benthic growths and detached masses in coastal areas. Toxic and tumor-promoting compounds characterized from benthic isolates include the phenolic bislactones aplysiatoxin and debromoaplysiatoxin and the indole alkaloids teleocidin, dihydroteleocidin and lyngbyatoxin A from *L. majuscula* (Fujiki et al., 1981; Moore, 1984). Isolates of *S. calcicola* and *O. nigroviridis* have also been reported to produce, respectively, debromoaphysiatoxin and another phenolic bislactone, oscillatoxin A (Moore, 1984; Carmichael, 1986, 1989).

Whether planktonic and particularly pelagic bloom-forming marine cyanobacteria are capable of toxin production is largely unknown. The most conspicuous example of this group, *Trichodesmium*, merits attention as a potential toxin-forming genus. Reports over several decades have implicated *Trichodesmium* in the deaths of fish, oysters and crabs and the avoidance of blooms by tuna fish (Chidambaram and Unny, 1944; Nagabhushanam, 1967; Chellam and Alagarswami, 1978). Other workers have not found grounds to suspect that *Trichodesmium* blooms may adversely affect marine zooplankton or macrofauna (Qasim, 1972; Devassy et al., 1979) and in the mortality reports it is not clear whether the animals died from physical blockage of the gills to deoxygenation of the water during bloom decay or to the presence of toxic compounds.

We have recently found that *Trichodesmium* bloom samples from the Caribbean are highly neurotoxic to mammals (Hawser et al., 1991). Here, we compare the toxicity of samples of *Trichodesmium* blooms collected in 1990 and 1991 in a range of bioassays and chemical procedures and discuss the significance of these findings in terms of the biology of *Trichodesmium* blooms.

2. Materials and Methods

2.1. BLOOM COLLECTIONS AND PREPARATION FOR TOXICITY ASSAYS

Trichodesmium spp. was collected at locations throughout the Caribbean during April 1990 and January-February 1991 during a research cruise on the RV Iselin with a 280 μm mesh, 1 m diameter plankton net. Samples were washed with freshly filtered sea-water, concentrated, frozen and freeze-dried. Prior to assay, freeze-dried bloom powder was suspended in MilliQ water and ultrasonicated for 6 x 15 sec bursts in

an MSE Soniprobe at full power with 15 sec rest periods in an ice-bath. The disrupted cell material was either tested directly in bioassays or centrifuged to provide a cell-free supernatant for assays.

2.2. MOUSE BIOASSAYS

Mouse bioassays were used to estimate and characterise the *in vivo* toxicity of the *Trichodesmium* bloom samples. Inbred Swiss BALBc female mice (18 to 25g) were injected intraperitoneally with *Trichodesmium* bloom samples in a 1 ml volume. The LD_{50} was estimated following doses at between 1000 and 1 mg dry wt per kg body wt and signs of toxicity and survival times noted.

2.3. BRINE SHRIMP ASSAY

A modified method of Harwig and Scott (1971) was used to determine bloom toxicity against the brine shrimp *Artemia salina*. Initial toxicity tests were carried out with up to 2.5 mg dry wt per ml and thereafter a double-dilution series of bloom extracts was used with up to 125 µg dry wt per ml in microtiter plates to determine the LC_{50}. Suspensions with 20-30 *A. salina* larvae in 100 µl and 100 µl of bloom extract or brine shrimp medium (controls) were added to wells. All treatments were carried out in triplicate. Plates were incubated at 28°C for 18 h. After incubations the percentage mortality of larvae relative to controls was determined.

2.4. *DAPHNIA* ASSAY

The freshwater invertebrates *Daphnia pulex* and *Daphnia magna* were used, neonates of both species being challenged with bloom extracts. In typical assays about 10 invertebrates in 800 µl medium were placed into wells of 24-well plates and allowed to equilibrate for 4 hours. Thereafter bloom extracts in 200 µl MilliQ water were added to treatment wells at 0-1000 µg/ml in triplicate. In controls 200 µl MilliQ was added in triplicate. Plates were incubated at 24°C for 24 h. After incubation the percentage mortalities of *Daphnia* spp. compared to controls were calculated.

2.5. MICROTOX ASSAY

Extracts of *Trichodesmium* spp. blooms were challenged against *Photobacterium phosphoreum* in Microtox bioassays as described previously (Lawton et al., 1990a). Bloom extracts were prepared at 5 mg dry wt per ml in Microtox diluent and tested at 2.25 mg/ml. Readings were recorded after 5 and 15 min to determine the EC_{50} value. All treatments were carried out in triplicate.

2.6. *IN VITRO* CHOLINESTERASE ASSAY

Samples of *Trichodesmium* blooms were tested against acetylcholinesterase (AChE; E.C. 3.1.1.7) from electric eel type V-S and butyrylcholinesterase (BUChE; E.C. 3.1.1.8) from horse serum. The

colourimetric method of Ellman *et al.* (1961) was used to determine enzyme activity. Bloom extracts were prepared as 10 mg dry wt per ml batches in 0.1 M potassium phosphate buffer (pH 8.0) and tested from 0 to 5 mg dry wt per ml. In control experiments 0.1 M KH_2PO_4 buffer was used only.

2.7. DIODE ARRAY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

Preliminary HPLC analyses were used to determine if the *Trichodesmium* bloom material contained known microcystins and nodularin (Lawton et al., 1990b) and the neurotoxin anatoxin-a (Carmichael, 1988). The column used was a 30DS reversed phase C18 run with a mobile phase gradient of 20-80% analytical grade methanol in MilliQ water and at a flow-rate of 1.0 ml/min. A Waters (Millipore) diode-array analyzer (model 991) was used for the analyses.

3. Results

The bloom sample collected in 1990 contained *Trichodesmium thiebautii* and *Trichodesmium erythraeum* with *T. thiebautii* predominating (Hawser et al., 1991). Extracts of this bloom were highly neurotoxic by mouse bioassay with signs of poisoning being typical of toxin(s) acting on the central nervous system: accelerated breathing rates, severe convulsions and death by respiratory arrest. Survival times at a dose of 100 mg dry wt per kg body wt were 2 to 5 minutes, and at the LD_{50} 15 to 30 minutes. Bloom samples consisting of *T. thiebautii* as the cyanobacterial component collected in 1991 showed the same signs of poisoning though with lower toxicities on a dry weight basis. *T. erythraeum*-dominated bloom samples have so far appeared to be non-toxic by mouse bioassay.

Table 1. Toxicity of *Trichodesmium* bloom samples to mice

Sample/Station	LD_{50}[a]	Survival Time
Trichodesmium spp./Station 17[b]	5	2-30 min
T. thiebautii/Station 14[c]	75	2-30 min
T. erythraeum/Station 14[c]	NT[d]	>24 hr
T. thiebautii/Station 15[c]	85	2-30 min
T. erythraeum/Station 15[c]	NT	>24 hr

a, intraperitoneal administration, values given in mg dry wt blooms sample per kg body wt; b, collected April 1990; c, collected January-February 1991; d, non-toxic at 100 mg/kg

The bloom extracts were also tested against *Artemia salina*. Initial tests were run at up to 2.5 mg dry wt per ml. At doses of 1.0 to 2.5 mg per ml 100% *A. salina* mortality was observed with the mixed *Trichodesmium* spp. sample and the *T. thiebautii* material after only 4 hours. A test range of 0-500 μg dry wt per ml was selected to determine LC_{50} values (Table 2).

Table 2. Toxicity of *Trichodesmium* bloom samples to the brine shrimp, *Artemia salina*.

Sample/Station	LC_{50} (μg dry wt per ml[a])
Trichodesmium spp./Station 17	30
T. thiebautii/Station 14	50
T. erythraeum/Station 14	NT[b]
T. thiebautii/Station 15	70
T. erythraeum/Station 15	NT

a, values are the mean of triplicate determinations
b, non-toxic at 1000 μg dry wt per ml over 18 hr

The *Trichodesmium* spp. mixed bloom and *T. thiebautii* extracts caused 100% mortality at doses of >100 μg per ml within 18 hr with LC_{50} values of 30 to 70. The *T. erythraeum* extracts appeared to have no adverse effects on the *A. salina* larvae at the high dose level of 1000 μg per ml over 18 hr. A second type of invertebrate toxicity assay used involved the freshwater fleas *Daphnia pulex* and *Daphnia magna*. The test range of both *Daphnia* spp. was 0 to 1000 μg dry wt per ml. The mixed *Trichodesmium* spp. bloom and *T. thiebautii* bloom extracts caused 100% mortalities within 24 hr in contrast to the *T. erythraeum* samples supplied at 1000 μg per ml. Both *Daphnia* species were sensitive to the *T. thiebautii* bloom samples although *D. pulex* was 3 to 6 times more sensitive than *D. magna*.

Microtox assays based on light emission by *Photobacterium phosphoreum* were used to determine the effects of *Trichodesmium* bloom extracts in a prokaryotic system. Extracts were tested at 2.25 mg dry wt per ml and resulted in significant decreases in light emission after 5 and 15 min, indicative of high toxicity (Table 4). As with the animal bioassays, the toxic effects on bacterial luminescence were caused by *T. thiebautii* bloom extracts, the high EC_{50} values for *T. erythraeum* samples being characteristic of non/low toxicity (see Lawton et al., 1990a).

Table 3. Toxicity of *Trichodesmium* bloom samples to two species of *Daphnia*

Sample/Station	LC_{50} (μg dry wt per ml[a])	
	D. pulex	*D. magna*
Trichodesmium spp./Station 17	12	70
T. thiebautii/Station 14	28	84
T. erythraeum/Station 14	>500	>500
T. thiebautii/Station 15	30	96
T. erythraeum/Station 15	>500	>500

a, values are the means of triplicate determinations

Table 4. Effects of *Trichodesmium* bloom samples on luminescence by *Photobacterium phosphoreum*

Sample/Station	EC_{50} (mg dry wt per ml)	
	5 min	15 min
Trichodesmium spp./Station 17	0.06	0.24
T. thiebautii/Station 14	0.13	0.30
T. erythraeum/Station 14	2.97	3.45
T. thiebautii/Station 15	0.17	0.32
T. erythraeum/Station 15	3.48	5.75

Diode array analysis was performed for the presence of known cyanobacterial toxins in the toxic *Trichodesmium* bloom extracts. No UV absorption peaks characteristic of peptide toxins or the alkaloid anatoxin-a were obtained. *In vitro* cholinesterase inhibition assays were used to determine if blooms contained anticholinesterase activity as found with a neurotoxic strain of *Anabaena flos-aquae* (Mahmood and Carmichael, 1987). No significant inhibition of cholinesterase was obtained with high levels of the *T. thiebautii* or *T. erythraeum* extracts, in contrast to a substantial inhibition by neurotoxic extracts of *Oscillatoria agardhii* used as a positive control (Table 5).

Table 5. Effects of *Trichodesmium* bloom extracts on cholinesterase activity *in vitro*

Sample/Station	% inhibition[a]	
	AChE[a]	BChE[c]
Trichodesmium spp./Station 17	3.5 + 1.0	3.0 + 1.0
T. thiebautii/Station 14	3.5 + 0.5	3.3 + 1.5
T. erythraeum/Station 14	2.75 + 1.0	1.95 + 1.0
T. thiebautii/Station 15	4.05 + 0.5	2.75 + 1.5
T. erythraeum/Station 15	3.75 + 1.0	3.5 + 1.5
Oscillatoria agardhii[d]	84.0 + 8.0	75.0 + 5.0

a, values are the means and standard errors of nine determinations; b, electric eel acetylcholinesterase type V-S; c, horse serum butyrylcholinesterase; d, S.P. Hawser and G.A. Codd, in preparation

4. Discussion

The similarity of many morphological and compositional features of *Trichodesmium* filaments to those of *Oscillatoria*, has led some phycologists to regard *Trichodesmium* species as members of an *Oscillatoria* grouping (see Fogg, 1982). These comparisons and the incidence of neurotoxin(s) and hepatotoxins among freshwater planktonic *Oscillatoria* spp. (Carmichael, 1988; Codd et al., 1989a,b; Sivonen et al., 1990) raise the possibility that *Trichodesmium* blooms may be toxic. The rapid appearance of neurotoxic symptoms in mice and low LD_{50}'s recorded with the 1990 *Trichodesmium* spp. bloom and 1991 *T. thiebautii* bloom samples clearly demonstrates the presence of toxic factor(s) associated with these blooms. The *T. thiebautii* samples were also toxic according to *Artemia, Daphnia* and *Photobacterium* assays in contrast to the *T. erythraeum* bloom extracts (Tables 2-4). These data suggest that the neurotoxicity of the 1990 mixed bloom of *T. thiebautii* plus *T. erythraeum* was not due to the latter *Trichodesmium* sp. although it is premature to infer to data that only *T. thiebautii* can be toxic.

Until the tests can be performed with cyanobacterial *Trichodesmium* isolates free from the heterotrophic bacteria associated with *Trichodesmium* filaments and aggregates (Paerl et al., 1989) it will not be possible to infer whether *Trichodesmium* sp. per se is neurotoxic. The inhibitory actions of the *T. thiebautii* bloom extracts against light emission by *P. phosphoreum* indicate that *Trichodesmium* bloom products may be active against prokaryotes and eukaryotes. Previous reports of antibacterial activity of *T. erythraeum* blooms exist (Ramamurthy and Krishnamurthy 1967; Ramamurthy, 1970)

emphasising the need to conduct toxicity studies with axenic *Trichodesmium* isolates.

Regardless of whether the toxic factor(s) are produced by the cyanobacterial or associated heterotrophic bacterial members of *Trichodesmium* bloom assemblages, the high toxicities found may be of biological and practical significance. The observations reported by Hawser et al. (1991) and here are not likely to be isolated findings of *Trichodesmium* bloom toxicity: of 89 *T. thiebautii*-dominated bloom samples collected on the January-February 1991 Iselin cruise, we have so far found about half of the samples to be toxic. Tests with 16 *T. erythraeum* bloom samples collected on the cruise have so are appeared to be non-toxic according to the same tests (S.P. Hawser and G.A. Codd unpublished observations). Although tests for known cyanobacterial neurotoxins have been negative e.g. lack of anatoxin-a by HPLC and of cholinesterase inhibition (Table 5), we have recently obtained neurotoxic diode array HPLC peaks from *T. thiebautii* bloom extracts. These components are being investigated.

The significance of the neurotoxic *Trichodesmium* bloom factor(s) may need to be taken into account in considering health aspects of consuming sea-food collected from areas with *Trichodesmium* blooms and human exposure to/contact with the blooms. Human health problems have been reported after contact with *T. erythraeum* blooms (Sato et al., 1966). As with freshwater cyanobacterial toxins (Lawton et al. 1990b), the biological significance of the *Trichodesmium* bloom toxin(s) requires investigation. Whether the toxins contribute to the avoidance of *Trichodesmium* blooms by fish and to fish and invertebrate kills is unknown (Chidambaram and Unny, 1944; Nagabhushanam, 1967; Chellam and Alagarswami, 1978). The ecological role of secondary metabolites of microbial origin, which we anthropocentrically term toxins, is a topic for consideration in the understanding of the dominance and "success" of cyanobacterial blooms. To the attributes of *Trichodesmium* blooms, for example in nitrogen fixation and buoyancy regulation, may need to be added the production of toxins (secondary metabolites) in accounting for the massive blooms of this conspicuous marine cyanobacterium.

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SUPEROXIDE DISMUTASE AS A PROTECTIVE ENZYME AGAINST OXYGEN TOXICITY: AN OVERVIEW AND INITIAL STUDIES IN *TRICHODESMIUM*

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ABSTRACT. The superoxide radical (O_2-), produced by the univalent reduction of molecular oxygen, is known to cause cellular damage due to its reactive nature. Superoxide dismutases (SODs) are a group of metalloenzymes which protect against the deleterious effects of superoxide by catalyzing its conversion to hydrogen peroxide and oxygen. Studies are underway to ascertain the role of SOD as a protective enzyme against oxygen toxicity in *Trichodesmium*. Initial experiments revealed that this organism's SOD is likely the iron-containing metallotype, as has been found in other cyanobacteria. Preliminary data also indicate that SOD activity varies in *Trichodesmium* on a diel basis, being the highest in the early morning, decreasing throughout the day, and reaching a low during the night.

1. Introduction

Although oxygen is a necessary element for all aerobic life, it has the potential to be toxic via the formation of oxygen radicals (Fridovich 1977, 1978). Oxygen radicals are too reactive to be well tolerated by living systems, and thus organisms have adapted means of protecting themselves from the toxic effects of reactive oxygen species. One type of oxygen radical, superoxide, can be enzymatically scavenged by the enzyme superoxide dismutase. The function of this enzyme was first described more than 20 years ago (McCord and Fridovich 1969), and it has since been isolated from a wide range of organisms.

Photosynthetic organisms, such as the cyanobacterium *Trichodesmium*, are particularly exposed to the hazards of oxygen toxicity owing to high internal concentrations of oxygen, as well as exposure to photooxidizing conditions. The aim of this review is to briefly summarize the literature on superoxide dismutase as an oxygen protective enzyme, particularly in nitrogen-fixing phototrophs, and to describe studies initiated to determine if superoxide dismutase is important in *Trichodesmium*.

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2. The Superoxide Radical

2.1 FORMATION OF SUPEROXIDE

Superoxide (O_2-) is an anionic oxygen radical produced by the univalent reduction of oxygen. In biological systems there are numerous reactions which produce substantial amounts of superoxide (Fridovich 1974, 1978). These reactions include spontaneous oxidation of hydroquinones, leukoflavins, catecholamines, thiols and reduced ferredoxins. Several enzymes, including xanthine oxidase, aldehyde oxidase, dihydro-orotic dehydrogenase and some flavin dehydrogenases also produce superoxide radicals.

During photosynthesis, superoxide and hydrogen peroxide are generated as a result of photoreduction of dioxygen. This process was first described in illuminated chloroplasts by Mehler (1951), and hence is termed a Mehler reaction. Subsequent studies have confirmed that both of these reactive oxygen species are generated by illuminated chloroplasts (Asada and Kiso 1973; Egneus et al. 1975). In addition to biological production of superoxide, aerobic irradiation of water by ionizing radiation or ultraviolet light can induce formation of this radical. The aquo electrons and hydrogen radicals formed during radiolysis or photolysis of water can univalently reduce oxygen to superoxide (Asada and Takahashi 1987).

2.2 DAMAGING EFFECTS OF SUPEROXIDE

The superoxide radical, which can act as either an oxidizing or a reducing agent, is part of a family of reactive oxygen species to which all aerobic organisms are exposed. The toxicity of oxygen radicals to biological systems has been well documented (Fridovich 1977, 1978, 1986a; Halliwell 1982; Salin 1987; Monk et al. 1989). The damaging effects of oxygen radicals to cellular components include denaturation of enzymes, depolymerization of polysaccharides, and DNA damage which results in genetic mutation. Lipid molecules are susceptible to oxygen radical induced peroxidation via the removal of hydrogen radicals, and such damage can lead to destruction of membrane integrity.

Although it has been suggested that superoxide is itself capable of mediating deleterious effects (Fridovich 1986a), this radical is also known to react with hydrogen peroxide and generate the more potent oxidizing agent, the hydroxyl radical (Haber and Weiss 1934). The hydroxyl radical is the most reactive species known to chemistry, and thus it is not surprising that the real danger of superoxide to cells is thought to lie in its ability to form hydroxyl radicals (Kong and Davison 1980; Halliwell 1982).

3. Superoxide Dismutase

3.1 CHARACTERISTICS AND DISTRIBUTION OF SUPEROXIDE DISMUTASE

Superoxide dismutase (SOD) is a superoxide scavenging enzyme which provides protection against oxygen radical mediated damage. The reaction catalyzed by SOD is the dismutation of superoxide to hydrogen peroxide and oxygen.

$$2(O_2^-) + 2(H^+) \longrightarrow H_2O_2 + O_2$$

SOD is a very efficient catalyst, operating close to the diffusion limit (Fridovich 1978).

Three distinct metallotypes of SOD have been described, each catalyzing the same reaction with comparable efficiency (Fridovich 1978). The SOD metallotypes can be distinguished by the metal group found at the active site. The description of the function of CuZn SOD, containing both copper and zinc at the active site, was the pioneering work for SOD research (McCord and Fridovich 1969). The CuZn SODs are unusually stable enzymes, retaining activity in 2% sodium dodecyl sulfate or in 8.0 M urea (Fridovich 1974, 1986b). Other SOD isozymes contain Fe (Fe SOD) or Mn (Mn SOD) at the active site. Based on amino acid sequence homologies, the Fe SOD and Mn SOD are closely related to each other, but are entirely different from CuZn SOD (Fridovich 1978).

SOD is a ubiquitous enzyme, being present in virtually all aerobic organisms and some anaerobic species. The general distribution pattern of SOD metallotypes indicates that CuZn SOD is primarily a eucaryotic enzyme, Fe SOD is a procaryotic enzyme and Mn SOD is found in procaryotes and in eucaryotic mitochondria (Bannister et al. 1987; Fridovich 1986b).

3.2 SUPEROXIDE DISMUTASE AND OXYGEN TOXICITY

The relationship between oxygen toxicity and SOD has been studied extensively, and several lines of evidence now exist to support the theory that SOD is an important enzyme in the protection against oxygen toxicity. Both *Escherichia coli* and *Streptococcus faecalis* cultures grown under increasing oxygen tensions expressed increased levels of SOD activity (Gregory and Fridovich 1973a), as did cultures of *Azotobacter vinelandii* (Dingler and Oelze 1987) and *Aquaspirillum magnetotacticum* (Short and Blakemore 1989). The specific activity of SOD was also enhanced in bacterial isolates from the high-dissolved-oxygen waters of Lake Hoare, Antarctica when cultured under high dissolved oxygen as compared to normal atmospheric oxygen conditions (Mikell et al. 1986). In cultures of the cyanobacterium *Anabaena cylindrica* incubated under variable oxygen tensions, SOD increased with increasing oxygen up to 60% pO_2 (Mackey and Smith 1983).

Not only is SOD activity enhanced in cultures grown at high oxygen tensions, but SOD levels have also been correlated with increased tolerance toward oxygen toxicity. *Escherichia coli* B cells containing increased levels of SOD after growth under 100%

oxygen were more resistant to the lethal effects of 20atm of oxygen than were cells with low, uninduced levels of SOD (Gregory and Fridovich 1973b). Similarly, in *Escherichia coli* growing in glucose-limited chemostat cultures, SOD activity increased with specific growth rate and was correlated with enhanced resistance to the lethal effects of hyperbaric oxygen (Hassan and Fridovich 1977).

3.3 SUPEROXIDE DISMUTASE AND NITROGENASE

Biological nitrogen fixation, the process of reducing dinitrogen to ammonia, is catalyzed by nitrogenase. Nitrogenase consists of two proteins, a MoFe protein and an Fe protein, both of which are readily susceptible to inactivation by oxygen (Postgate 1982). Hence it is of interest to determine how photosynthetic diazotrophs, such as *Trichodesmium,* maintain nitrogenase activity in an aerobic environment with additional photosynthetically generated oxygen. Diazotrophs have evolved a variety of means for protecting nitrogenase from oxygen inactivation; however the oxygen protective system employed by *Trichodesmium* has yet to be elucidated (Carpenter et al. 1990). Aspects of the protection of nitrogenase from oxygen inactivation are discussed in further detail elsewhere (Gallon and Stal, this volume; Paerl and Bebout this volume).

Due to the toxic nature of both superoxide and the reactive oxygen species it generates, it is not surprising that SOD has been implicated in the protection of nitrogenase from oxygen mediated damage (Becana and Rodriguez-Barrueco 1989). Specifically, it has been noted that the SOD content of free-living nitrogen-fixing bacteria is often significantly higher than that of non-diazotrophs (Puppo and Riguad 1986). In cultures of *Frankia*, a diazotrophic actinomycete, Steele and Stowers (1986) reported that under non-nitrogen fixing conditions SOD (MnSOD) activity is among the highest in procaryotes. In the same study, derepression for nitrogen fixation in *Frankia* resulted in a several-fold increase in both SOD and catalase activities, as well as induction of a second SOD isozyme (FeSOD). Tozum and Gallon (1979) have observed that maximum nitrogenase activity in cultures of the cyanobacterium *Gloeocapsa* coincides with maximum activity of several oxygen-protective enzymes, including SOD. Studies on the effects of high oxygen tensions in the cyanobacterium *Anabaena cylindrica* revealed that this organism can adapt to protect its nitrogenase against oxygen inactivation, and that this adaptive process is correlated with induction of SOD and catalase (Mackey and Smith 1983). In studies on oxygen toxicity in the heterotrophic diazotroph *Azotobacter chroococcum*, it was found that superoxide exogenously supplied to nitrogen-fixing whole cells inhibited nitrogenase activity, and that addition of SOD to samples before superoxide generation reversed the inhibition of nitrogenase (Buchanan 1977).

It has been postulated that *Trichodesmium* may employ SOD as a protective mechanism against oxygen inactivation of nitrogenase (Taylor pers. comm.), however no confirmatory evidence has yet

been gathered. Acquiring such evidence is one goal of the current study.

3.4 SUPEROXIDE DISMUTASE AND PHOTOADAPTATION

Several factors put photosynthetic organisms at particular risk from the formation of reactive oxygen species. These include high internal oxygen concentrations as a result of photosynthetic oxygen evolution, photoreduction of dioxygen to superoxide (Mehler reaction), and the presence of pigments which participate in photosensitized reactions generating singlet oxygen and possibly hydroxyl radicals (Asada and Takahashi 1987: Halliwell 1982: Salin 1987). Thus, enzymes such as SOD which participate in scavenging reactive oxygen species should be important in the photoadaptive response of organisms, such as *Trichodesmium*, exposed to high light regimes.

Several recent studies have demonstrated such a relationship. SOD has been shown to increase with irradiance and in response to ultraviolet light in zooxanthellae from the sea anemone *Aiptasia pallida* (Lesser and Shick 1989a). In a similar study, not only did SOD activity in zooxanthellae correlate with irradiance, but when individual anemones were reciprocally transplanted from bright and dim areas, a reversal of SOD activity in the symbiotic zooxanthellae occurred (Lesser and Shick 1989b). SOD activity was also found to vary directly with irradiance in the symbiotic procaryote *Prochloron*, being significantly higher in samples collected at a depth of 3m in comparison to samples from 17m at the Great Barrier Reef, Australia (Lesser and Stochaj 1990). In a study investigating the effects of photooxidative conditions on cyanobacteria, a pond bloom isolate of *Microcystis* which exhibited extremely high resistance to photooxidative damage was found to have stable SOD levels under conditions of intense illumination (Eloff et al. 1976). Interestingly, exposure of this *Microcystis* isolate to chloramphenicol, a protein synthesis inhibitor, resulted in decreased SOD activity and coincided with a loss in resistance to photooxidation.

4. The Role of Superoxide Dismutase in *Trichodesmium*

4.1 *TRICHODESMIUM* AND PHOTOTOXIC EFFECTS

Trichodesmium inhabits an extreme environment with respect to photooxidative conditions. This pelagic cyanobacterium is found in the open ocean, primarily at tropical and subtropical latitudes, and is most abundant in the upper 25 meters (Carpenter and McCarthy 1975; Carpenter and Price 1977). That *Trichodesmium* frequently occur in blooms is well documented (Bowman and Lancaster 1965; Carpenter 1983; Creagh 1985; Carpenter and Capone, this volume). Furthermore, *Trichodesmium* blooms often occur when there is little or no cloud cover (Creagh 1985; McCarthy and Carpenter 1979), thus exposing the organisms to potential photooxidative damage.

Because *Trichodesmium* grow at or near the surface, they may be

exposed to superoxide formed from photoreduction of dioxygen (Mehler activity). See Kana, this volume, for a detailed discussion on Mehler activity in Trichodesmium. Reactive oxygen species may also be generated in *Trichodesmium* as a consequence of photosensitized reactions or aerobic irradiation of water by ultraviolet light (see above). Ultraviolet radiation (280-400nm) is now known to penetrate to depths of at least 10m in seawater (Smith and Baker 1979; Calkins and Thordardottir 1980; Wood 1987), and can thus contribute to phototoxic reactions even in *Trichodesmium* growing below the surface.

Several studies have investigated the effects of high light intensities on *Trichodesmium*. McCarthy and Carpenter (1979) and Mague et al. (1977) have both shown that high light depresses photosynthesis in *Trichodesmium*. In contrast, Mague et al. (1977) found no evidence of photoinhibition of nitrogen fixation in *Trichodesmium* incubated under light intensities ranging from 0 to 97% transmission of incident light.

4.2 MEASUREMENT OF SUPEROXIDE DISMUTASE IN *TRICHODESMIUM*

Clearly, *Trichodesmium* must protect its nitrogenase as well as other cellular components from the damaging effects of reactive oxygen species. The possibility that SOD is important in preventing the lethal effects of active oxygen in *Trichodesmium* is the goal of studies in progress at the time of this writing.

SOD has been assayed on cell-free extracts of *Trichodesmium thiebautii* collected from the Bahamas and Eastern Caribbean. A spectrophotometric assay originally described by Elstner and Heupel (1976) and modified by Oyanagui (1984) was used to measure SOD activity. This assay is based on the ability of SOD to inhibit the superoxide-induced oxidation of hydroxylamine to nitrite.

The three metallotypes of SOD (CuZn, Fe, and Mn) can be distinguished by their differential susceptibility to inhibition by cyanide and hydrogen peroxide. CuZn and Fe SOD are inhibited by hydrogen peroxide; only CuZn SOD is inhibited by cyanide; and Mn SOD is resistant to both inhibitors (Bannister et al. 1987). *Trichodesmium* SOD was found to be insensitive to cyanide, but completely inactivated by hydrogen peroxide, thus demonstrating the presence of an Fe SOD (unpublished data). This would be expected, as Fe SOD is the metallotype typically found in cyanobacteria (Cseke et al. 1979; Bannister et al. 1987).

To ascertain whether SOD activity varies in *Trichodesmium* on a diel basis, assays were conducted on samples collected over 24 hour periods. These experiments revealed a diel SOD activity pattern in *Trichodesmium*, with activity being the highest in the early morning, decreasing throughout the day, and reaching a low during the night (unpublished data). It is not yet clear what controls this observed cycle, or if these diel changes in SOD activity are connected with the diel patterns of nitrogenase activity in *Trichodesmium* (Capone et al. 1990). This will be the focus of future research.

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TRICHODESMIUM BLOOMS IN THE GULF OF THAILAND

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ABSTRACT. This study reviews data collected during *Trichodesmium* blooms in the Gulf of Thailand. The blooms appear to be harmless events, but under exceptional conditions, *Trichodesmium* are driven inshore by tides and winds and have caused extensive damage to farmed fish and shrimp. Blooms have been recently observed in wider areas than that observed during the last decade. *Trichodesmium* is a source of food for zooplankton and plankton feeding fish. Relationships between *Trichodesmium* blooms and environmental data are examined and discussed.

1. INTRODUCTION

Trichodesmium blooms have been recorded in the last 30 years as a natural phenomenon in the Gulf of Thailand. Most early observations were concerned with the impact on the fisheries industry (Charernpol, 1957). The distribution of *Trichodesmium* in the West Pacific region was reported by Marumo, (1973), and red tides were observed to be formed by this cyanobacterium in the south China Sea, Sulu Sea, east of Sumatra, south of Timor, near Palawan Island and east of North Borneo. A bloom in Hong Kong harbor was recorded by Lam (1984), one in Jakarta Bay by Adnon (1984), and others in the west coast of India by Devassy (1989). In the Gulf of Thailand, *Trichodesmium* was abundant and occurred in nearly all collecting stations and was recorded as the major species of phytoplankton usually found during red tides in offshore waters (Suvapepun 1982). In blooms the organisms accumulate at the surface and are driven by tide and wind in bands of yellowish-green or brown water of several miles extent. The blooms appear to be harmless events, but under exceptional conditions the algae have been driven ashore by the wind causing problems to coastal aquaculture activities.

2. LONG TERM MONITORING

Oceanographic studies in the Gulf of Thailand have been carried out by the Marine Fisheries Division, Department of Fisheries since 1971, continuously up to the present time. The red tide causative organism normally was *Trichodesmium erythraeum*. Blooms have been recently observed in wider areas, and the frequency of occurrence has fluctuated during the last decade (Fig. 1). *Trichodesmium* blooms usually occur from January to July, with greatest abundance during the warmest months of the year in April and May. There were 51 occurrences of blooms detected in the Gulf of Thailand from 1981 to 1990. Average number of trichomes during the discolored water in May 1983 was 5.7 million trichomes liter^{-1} of surface water. During normal blooms, the number was from 176,000 to 314,000 trichomes liter^{-1} of surface water.

E. J. Carpenter et al. (eds.), Marine Pelagic Cyanobacteria: Trichodesmium and other Diazotrophs, 343–348.
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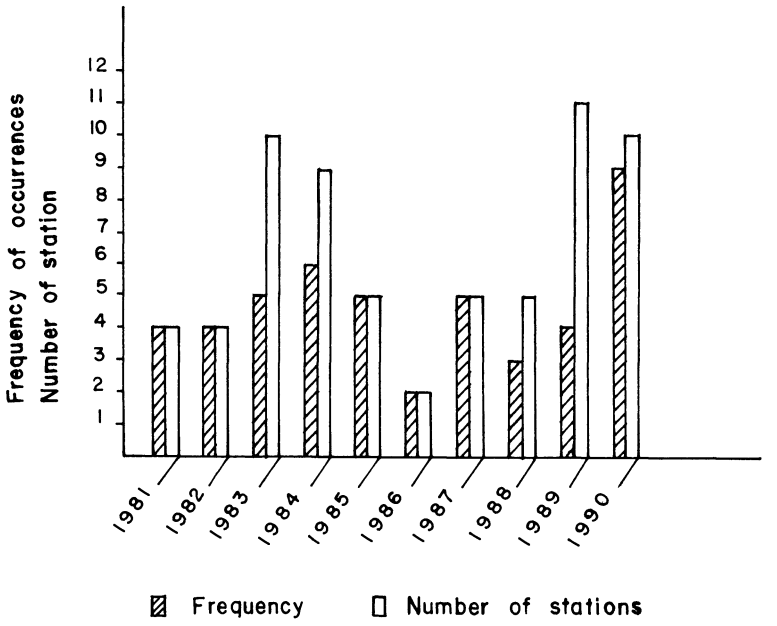

Fig. 1 Occurrences and number of stations affected by Trichodesmium during 1981 – 1990.

Trichodesmium was generally concentrated in the upper 20 meters, and concentration decreased remarkably with depth below 5 meters from the surface. *Trichodesmium erythraeum* usually appears in plankton samples throughout the water column in small clusters of trichomes. During the bloom trichomes form a raft-like colony, and number of trichomes in one colony was from 27-115. The colony normally detected with the naked eye was 2-3 mm long. Trichomes usually appeared free in offshore water throughout the year, but during the bloom trichomes massed together and rose to the surface layer and caused the water to appear yellow in color at the start of the bloom and change to gray, yellow-green, yellow-brown and brown or reddish-brown during later bloom stages.

3. PROBLEMS CAUSED BY *TRICHODESMIUM*

A large bloom of *Trichodesmium erythraeum* was detected on 11 May 1983 scattered over a wide area of approximately 7000 km^2. The discolored water spread from the east coast to the north into the Inner Gulf and remained until 14 June 1983. The organisms accumulated at the offshore sea surface and were driven by wind into bands of yellow-green water several miles in extent. In

June the bloom was driven to the shore, and water became reddish-brown along a stretch of almost 27 km. When washed ashore *Trichodesmium* accumulated into reddish-brown foam patches in the surf of a sandy beach about 20 km long. This bloom caused extensive damage to fish farms on the east coast, and estimated loss was about U.S. $ 1.16 million (Suvapepun *et al.* 1984), but no harmful effects on fish were observed in the sea. Recent blooms of *Trichodesmium* in March 1991 caused mass mortalities of shrimp cultured in ponds along the east coast, and neither fish nor other mortality as a result of this bloom were observed or reported from the surrounding areas. Shrimp culture along the southern coast of the Gulf has become an important industry, but in recent years mass mortalities of farmed shrimp have been associated with blooms of *Trichodesmium.* It is not clear whether the mass mortality of farmed fish and shrimp were due to toxin release by *Trichodesmium* into the surrounding water or due to lack of oxygen in the water. Toxicity studies on white sea bass (*Lates calcarifer*) were carried out. Thirty percent of fish larvae exposed to water from a natural bloom of *Trichodesmium* were killed within 24 hours when the density of algae exceed 691,500 trichomes liter^{-1} at 29°C, pH 8.3, NH_4 1.54 mg liter^{-1}, NO_3 1.46 mg liter^{-1} (Suvapepun *et al.* 1984).

4. POSITIVE EFFECT ON BLOOMS

Not all blooms have deleterious effects. On the positive side, the alga is a source of food for zooplankton and plankton feeding fish. Lursinsap (1967) studied the stomach contents of Indo-Pacific mackerel (*Rastrelliger neglectus*) and she found *Trichodesmium* comprised the major food items of the fish. The percentage of *Trichodesmium* in stomach contents of *Rastrelliger* observed in April 1964 was 72%. During routine studies on red tide species, *Trichodesmium* cells were found within the body of Tintinnids in the samples.

5. CAUSE OF EXCEPTIONAL BLOOMS

Investigations of red tides were carried out in the coastal area of the Gulf of Thailand by the Marine Fisheries Division, Department of Fisheries. Monthly surveys for red tides were conducted during the period from 1981-1991 by research vessels. *Trichodesmium* blooms typically were recorded from January to September. Most of the incidents occurred in March-April and July. Blooms rarely appeared during the northeast monsoon months from October through January. Water quality monitoring data collected during the same cruises were used to identify conditions which induce red tide formation.

The environmental conditions which were associated with the *Trichodesmium* blooms varied. Water temperature was in the range 27.0° - 31.5°C, pH 7.35 - 8.45, salinity 31.2 - 33.0 ppt, dissolved oxygen 3.5 - 5.98 ml liter^{-1}, nitrate 0.13 - 11.9 µg-at-N liter^{-1}, phosphate 0.09 - 2.0 µg-at-P liter^{-1}. It is difficult to define the factors responsible for bloom development from the existing data. Nutrient concentrations, salinity and surface water temperature in areas affected by *Trichodesmium* blooms in 1985 (Fig. 2 and 3) indicated the association of blooms with high levels of nitrate. For example, the blooms in March 1985 at station 58 and 62 coincided with the high concentration of nitrate. Blooms in July 1985 at station 66 and 75 occurred where the concentration of nitrate reached highest peak at 11.5 and 11.8 µg-at liter^{-1}. Water temperature, salinity and oceanographic changes during the two monsoons in the Gulf of Thailand might be related to *Trichodesmium* blooms, and this is not well explained based on the existing data.

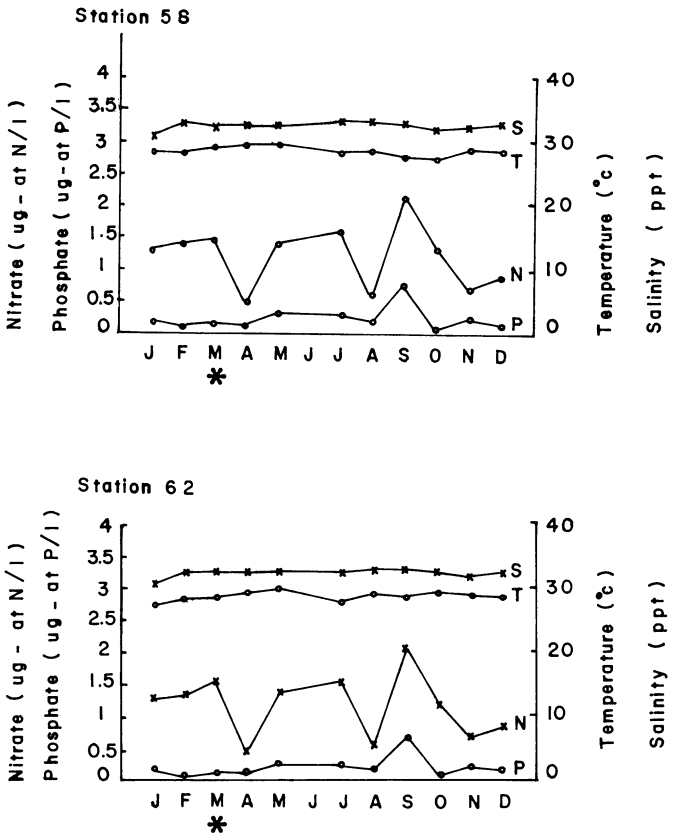

Figure 2. Monthly distribution of temperature, salinity, nitrate and phosphate at Station 58 and 62 in 1985. A *Trichodesmium* bloom (*) occurred in March 1985.

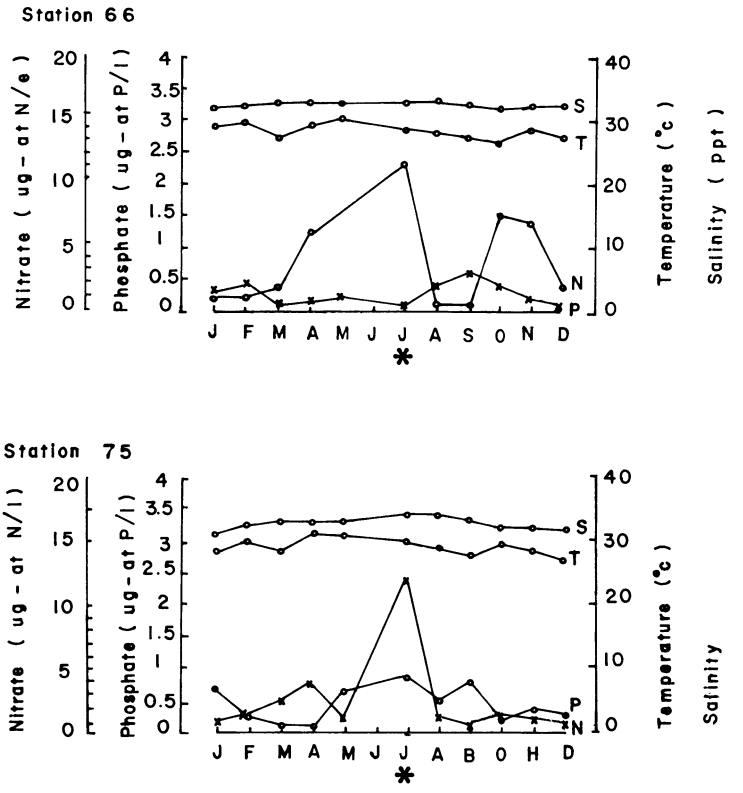

Figure 3. Monthly distribution of temperature, salinity, nitrate and phosphate at station 66 and 75 in 1985. A Trichodesmium bloom (*) occurred in July 1985.

In summary, blooms of *Trichodesmium* are common in the Gulf of Thailand, especially between January and September when water temperature exceeds 27°C. Blooms can reach 7000 km^2 in area and are occasionally toxic to fish and shrimp; however, the cause of the mortality requires further investigation. *Trichodesmium* appears to be a major food item for some species of fish and perhaps for protozoa such as Tintinnids. *Trichodesmium* appears to be very significant in the Gulf of Thailand ecosystem.

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